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(54) Title: NUCLEIC ACID SEQUENCES AND METHODS FOR THE MODIFICATION OF PLANT GENE EXPRESSION

(57) Abstract: Novel isolated plant polynucleotide promoter sequences are provided, together with genetic constructs comprising such polynucleotides. Methods for using such constructs in modulating the transcription of DNA sequences of interest are also disclosed, together with transgenic plants comprising such constructs.

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Nucleic acid sequences and methods for the modification of plant gene expression

Technical Field of the Invention

This invention relates to the regulation of polynucleotide transcription and/or expression. More specifically, this invention relates to polynucleotide regulatory sequences isolated from plants that are capable of initiating and driving the transcription of polynucleotides, and the use of such regulatory sequences in the modification of transcription of endogenous and/or heterologous polynucleotides and production of polypeptides. Polypeptide sequences are also disclosed.

Background of the Invention

Gene expression is regulated, in part, by the cellular processes involved in transcription. During transcription, a single-stranded RNA complementary to the DNA sequence to be transcribed is formed by the action of RNA polymerases. Initiation of transcription in eukaryotic cells is regulated by complex interactions between *cis*-acting DNA motifs, located within the gene to be transcribed, and *trans*-acting protein factors. Among the *cis*-acting regulatory regions are sequences of DNA, termed promoters, to which RNA polymerase is first bound, either directly or indirectly. As used herein, the term "promoter" refers to the 5' untranslated region of a gene that is associated with transcription and which generally includes a transcription start site. Other cis-acting DNA motifs, such as enhancers, may be situated further up- and/or down-stream from the initiation site.

Both promoters and enhancers are generally composed of several discrete, often redundant elements, each of which may be recognized by one or more *trans*-acting regulatory proteins, known as transcription factors. Promoters generally comprise both proximal and more distant elements. For example, the so-called TATA box, which is important for the binding of regulatory proteins, is generally found about 25 basepairs upstream from the initiation site. The so-called CAAT box is generally found about 75 basepairs upstream of the initiation site. Promoters generally contain between about 100 and 1000 nucleotides, although longer promoter sequences are possible.

For the development of transgenic plants, constitutive promoters that drive strong transgene expression are preferred. Currently, the only available constitutive plant promoter

that is widely used is derived from Cauliflower Mosaic Virus. Furthermore, there exists a need for plant-derived promoters for use in transgenic food plants due to public conceptions regarding the use of viral promoters. Few gymnosperm promoters have been cloned and those derived from angiosperms have been found to function poorly in gymnosperms. There thus remains a need in the art for polynucleotide promoter regions isolated from plants for use in modulating transcription and expression of polynucleotides in transgenic plants.

Summary of the Invention

Briefly, isolated polynucleotide regulatory sequences from eucalyptus and pine that are involved in the regulation of gene expression are disclosed, together with methods for the use of such polynucleotide regulatory regions in the modification of expression of endogenous and/or heterologous polynucleotides in transgenic plants. In particular, the present invention provides polynucleotide promoter sequences from 5' untranslated, or non-coding, regions of plant genes that initiate and regulate transcription of polynucleotides placed under their control, together with isolated polynucleotides comprising such promoter sequences.

In a first aspect, the present invention provides isolated polynucleotide sequences comprising a polynucleotide selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; (b) complements of the sequences recited in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; (c) reverse complements of the sequences recited in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; (d) reverse sequences of the sequences recited in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; (e) sequences having either 40%, 60%, 75% or 90% identical nucleotides, as defined herein, to a sequence of (a) – (d); probes and primers corresponding to the sequences set out in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; polynucleotides comprising at least a specified number of contiguous residues of any of the polynucleotides identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; and extended sequences comprising portions of the sequences set out in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; all of which are referred to herein as "polynucleotides of the present invention." The present invention also provides isolated polypeptide sequences identified in the attached Sequence Listing as SEQ ID NO: 63-80, 87 and 130; polypeptide variants of those sequences; and polypeptides comprising the isolated polypeptide sequences and variants of those sequences.

In another aspect, the present invention provides genetic constructs comprising a polynucleotide of the present invention, either alone, or in combination with one or more additional polynucleotides of the present invention, or in combination with one or more known polynucleotides, together with cells and target organisms comprising such constructs.

In a related aspect, the present invention provides genetic constructs comprising, in the 5'-3' direction, a polynucleotide promoter sequence of the present invention, a polynucleotide to be transcribed, and a gene termination sequence. The polynucleotide to be transcribed may comprise an open reading frame of a polynucleotide that encodes a polypeptide of interest, or it may be a non-coding, or untranslated, region of a polynucleotide of interest. The open reading frame may be orientated in either a sense or antisense direction. Preferably, the gene termination sequence is functional in a host plant. Most preferably, the gene termination sequence is that of the gene of interest, but others generally used in the art, such as the *Agrobacterium tumefaciens* nopalin synthase terminator may be usefully employed in the present invention. The genetic construct may further include a marker for the identification of transformed cells.

In a further aspect, transgenic plant cells comprising the genetic constructs of the present invention are provided, together with organisms, such as plants, comprising such transgenic cells, and fruits, seeds and other products, derivatives, or progeny of such plants. Propagules of the inventive transgenic plants are included in the present invention. As used herein, the word "propagule" means any part of a plant that may be used in reproduction or propagation, sexual or asexual, including cuttings.

Plant varieties, particularly registerable plant varieties according to Plant Breeders' Rights, may be excluded from the present invention. A plant need not be considered a "plant variety" simply because it contains stably within its genome a transgene, introduced into a cell of the plant or an ancestor thereof.

In yet another aspect, methods for modifying gene expression in a target organism, such as a plant, are provided, such methods including stably incorporating into the genome of the organism a genetic construct of the present invention. In a preferred embodiment, the target organism is a plant, more preferably a woody plant, most preferably selected from the group consisting of eucalyptus and pine species, most preferably from the group consisting of *Eucalyptus grandis* and *Pinus radiata*.

In another aspect, methods for producing a target organism, such as a plant, having modified polypeptide expression are provided, such methods comprising transforming a plant

cell with a genetic construct of the present invention to provide a transgenic cell, and cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth.

In other aspects, methods for identifying a gene responsible for a desired function or phenotype are provided, the methods comprising transforming a plant cell with a genetic construct comprising a polynucleotide promoter sequence of the present invention operably linked to a polynucleotide to be tested, cultivating the plant cell under conditions conducive to regeneration and mature plant growth to provide a transgenic plant; and comparing the phenotype of the transgenic plant with the phenotype of non-transformed, or wild-type, plants.

In yet a further aspect, the present invention provides isolated polynucleotides that encode ubiquitin. In specific embodiments, the isolated polynucleotides comprise a polynucleotide selected from the group consisting of: (a) sequences recited in SEQ ID NO: 1 and 34; (b) complements of the sequences recited in SEQ ID NO: 1 and 34; (c) reverse complements of the sequences recited in SEQ ID NO: 1 and 34; (d) reverse sequences of the sequence recited in SEQ ID NO: 1 and 34; and (e) sequences having either 40%, 60%, 75% or 90% identical nucleotides, as defined herein, to a sequence of (a) – (d). Polypeptides encoded by such polynucleotides are also provided, together with genetic constructs comprising such polynucleotides, and host cells and transgenic organisms, for example plants, transformed with such genetic constructs. In specific embodiments, such polypeptides comprise a sequence provided in SEQ ID NO: 80 or 67.

In yet further aspects, the present invention provides isolated polynucleotides comprising the DNA sequence of SEQ ID NO: 21, or a complement, reverse complement or variant of SEQ ID NO: 21, together with genetic constructs comprising such polynucleotides and cells transformed with such sequences. As discussed below, removal of the sequence of SEQ ID NO: 21 from a polynucleotide that comprises the sequence of SEQ ID NO: 21 may enhance expression of the polynucleotide. Conversely, the inclusion of the sequence of SEQ ID NO: 21 in a genetic construct comprising a polynucleotide of interest may decrease expression of the polynucleotide.

The above-mentioned and additional features of the present invention and the manner of obtaining them will become apparent, and the invention will be best understood by reference to the following more detailed description. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

Brief Description of the Drawings

Fig. 1 shows the expression in *A. thaliana* of the GUS gene in promoter reporter constructs containing either the superubiquitin promoter with introns, the superubiquitin promoter without introns, or the CaMV 35S promoter. The GUS expression was measured by fluorimetric determination of 4-methyl-umbelliferone (MU) in protein extracts from these plants.

Fig. 2 shows the expression of the GUS gene in tobacco plant protoplasts by deletion constructs containing the superubiquitin promoter with or without the intron. The constructs contained 1,103; 753; 573; 446; 368; and 195 bp upstream of the TATA sequence (bp numbers 1,104-1,110 of SEQ ID NO: 2). The GUS expression was measured by fluorimetric determination of 4-methyl-umbelliferone (MU) in protein extracts from these protoplasts.

Fig. 3 shows the expression of the GUS gene in tobacco plant protoplasts by constructs containing *P. radiata* either the constitutive promoters Elongation factor-1 alpha, 5-adenosylmethionine synthetase or the superubiquitin promoter without the intron. The GUS expression was measured by fluorimetric determination of 4-methyl-umbelliferone (MU) in protein extracts from these protoplasts.

Fig. 4 shows the expression of the GUS gene in tobacco plant protoplasts by a deletion construct containing a fragment of the *E. grandis* constitutive promoter Elongation factor-1 alpha.

Fig. 5 shows the expression in *A. thaliana* of the GUS gene in promoter reporter constructs containing the 3' UTR of the superubiquitin promoter in sense or antisense orientation together with either the superubiquitin promoter with intron, the superubiquitin promoter without intron, or the CaMV 35S promoter. The GUS expression was measured by fluorimetric determination of 4-methyl-umbelliferone (MU) in protein extracts from these plants.

Detailed Description of the Invention

The present invention provides isolated polynucleotide regulatory regions that may be employed in the manipulation of plant phenotypes, together with isolated polynucleotides comprising such regulatory regions. More specifically, polynucleotide promoter sequences isolated from pine and eucalyptus are disclosed. As discussed above, promoters are components of the cellular "transcription apparatus" and are involved in the regulation of

gene expression. Both tissue- and temporal-specific gene expression patterns have been shown to be initiated and controlled by promoters during the natural development of a plant. The isolated polynucleotide promoter sequences of the present invention may thus be employed in the modification of growth and development of plants, and of cellular responses to external stimuli, such as environmental factors and disease pathogens.

Using the methods and materials of the present invention, the amount of a specific polypeptide of interest may be increased or reduced by incorporating additional copies of genes, or coding sequences, encoding the polypeptide, operably linked to an inventive promoter sequence, into the genome of a target organism, such as a plant. Similarly, an increase or decrease in the amount of the polypeptide may be obtained by transforming the target plant with antisense copies of such genes.

The polynucleotides of the present invention were isolated from forestry plant sources, namely from *Eucalyptus grandis* and *Pinus radiata*, but they may alternatively be synthesized using conventional synthesis techniques. Specifically, isolated polynucleotides of the present invention include polynucleotides comprising a sequence selected from the group consisting of sequences identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; complements of the sequences identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; reverse complements of the sequences identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; at least a specified number of contiguous residues (*x*-mers) of any of the above-mentioned polynucleotides; extended sequences corresponding to any of the above polynucleotides; antisense sequences corresponding to any of the above polynucleotides; and variants of any of the above polynucleotides, as that term is described in this specification.

In another embodiment, the present invention provides isolated polypeptides encoded by the polynucleotides of SEQ ID NO: 63-80, 87 and 130.

The polynucleotides and polypeptides of the present invention were putatively identified by DNA and polypeptide similarity searches. In the attached Sequence Listing, SEQ ID NOS. 1-14, 20, 22-62, 81-86 and 88-127 are polynucleotide sequences, and SEQ ID NOS. 63-80, 87 and 130 are polypeptide sequences. The polynucleotides and polypeptides of the present invention have demonstrated similarity to promoters that are known to be involved in regulation of transcription and/or expression in plants. The putative identity of each of the inventive polynucleotides is shown below in Table 1, together with the 5' untranslated region (5' UTR) or putative promoter region (identified by residue number).

TABLE 1

Polynucleotide SEQ ID NO:	Polypeptide SEQ ID NO:	5' UTR	IDENTITY
1	80	1-2064	Super Ubiquitin coding region and UTRs
2	-	1-2064	Super Ubiquitin promoter with intron
3	-	1-1226	Super Ubiquitin promoter without intron
4	-	1-431	Cell division control
5	-	1-167	Xylogenesis – specific
6	-	1-600	4-Coumarate-CoA Ligase (4CL)
7	-	1-591	Cellulose synthase
8	-	1-480	3' end, Cellulose synthase
20	-	1-363	5' end, Cellulose synthase
9	-	1-259	Leaf specific
10	-	1-251	Leaf specific
11	-	1-248	Leaf specific
12	-	1-654	O-methyl transferase
13	-	1-396	Root specific
14	-	1-763	Root specific
22	63	1-406	Pollen coat protein
23	-	1-350	Pollen allergen
24	-	1-49	Pollen allergen
25	64	1-284	Pollen allergen
26	65	1-77	Auxin-induced protein
27	-	1-74	Auxin-induced protein
28	66	1-99	Auxin-induced protein
29	-	1-927	Flower specific
30	-	1-411	Flower specific
31	-	1-178	Flower specific

Polynucleotide SEQ ID NO:	Polypeptide SEQ ID NO:	5' UTR	IDENTITY
32	-	1-178	Flower specific
33	-	1-178	Flower specific
34	67	1-805	Ubiquitin
35	68	1-81	Glyceraldehyde-3-phosphate dehydrogenase
36	69	1-694	Carbonic anhydrase
37	-	1-648	Isoflavone reductase
38	-	1-288	Isoflavone reductase
39	-	1-382	Glyceraldehyde-3-phosphate dehydrogenase
40	70	1-343	Bud specific
41	-	1-313	Xylem-specific
42	-	1-713	Xylem-specific
43	-	1-28	Xylem-specific
44	-	1-35	Xylem-specific
45	71	1-180	Meristem-specific
46	72	1-238	Senescence-like protein
47	-	1-91	Senescence-like protein
48	-	1-91	Senescence-like protein
49	-	1-809	Pollen-specific
50	-	1-428	Pollen-specific
51	73	1-55	Pollen-specific
52	74	1-575	Pollen-specific
53	75	1-35	Pollen-specific
54	-	1-335	Nodulin homolog pollen specific
55	-	1-336	Nodulin homolog pollen specific
56	76	1-157	Sucrose synthase
57	77	1-446	Sucrose synthase
58	-	1-326	Sucrose synthase

Polynucleotide SEQ ID NO:	Polypeptide SEQ ID NO:	5' UTR	IDENTITY
59	-	1-311	Flower specific
60	78	1-694	O-methyl transferase
61	79	1-112	Elongation factor A
62	-	1-420	Elongation factor A
81	-	-	MIF homologue
82	-	-	MIF homologue
83	-	-	MIF homologue
84	-	-	MIF homologue
85	-	-	MIF homologue
86	87	1-87	MIF homologue
88	-	1-1156	Chalcone synthase
89	-	1-2590	Unknown flower specific
90	-	1-1172	Unknown flower specific
91	-	1-446	Sucrose synthase
92	-	1-2119	Unknown xylem specific
93	-	1-2571	Glyceraldehyde-3-Phosphate dehydrogenase
94	-	1-1406	Unknown pollen specific
95	-	1-2546	*Pinus radiata* male-specific protein (PrMALE1)
96	-	1-4726	*Pinus radiata* male-specific protein (PrMALE1)
97	-	1-635	UDP glucose glycosyltransferase
98	-	1-468	Elongation Factor A1
99	-	1-222	Elongation Factor A1
100	-	1-410	S-adenosylmethionine synthetase
101	-	1-482	S-adenosylmethionine synthetase
102	-	1-230	S-adenosylmethionine synthetase
103	-	1-596	UDP glucose 6 dehydrogenase
104	-	1-653	Hypothetical protein

Polynucleotide SEQ ID NO:	Polypeptide SEQ ID NO:	5' UTR	IDENTITY
105	-	1-342	Laccase 1
106	-	1-342	Laccase 1
106	-	1-948	Arabinogalactan-like 1
108	-	1-362	Arabinogalactan-like 2
109	-	1-326	Arabinogalactan like-2
110	-	1-296	Root Receptor-like kinase
111	-	1-723	Root Receptor-like kinase
112	-	1-1301	*Pinus radiata* Lipid Transfer Protein 2 (PrLTP2)
113	-	1-1668	Caffeic acid O-methyltransferase
114	-	1-850	UDP glucose glycosyltransferase
115	-	1-986	UDP glucose 6 dehydrogenase
116	-	1-947	Laccase 1
117	-	1-1766	Arabinogalactan like-1
118	-	1-1614	Constans
119	-	1-602	Flowering Promoting Factor 1 (FPF1)
120	-	1-901	Agamous
121	-	1-1,245	Dreb 1A Transcription factor
122	-	1-959	Drought Induced Protein 19
123	-	1-1,140	Salt Tolerance protein
124	130	1-887	Low Temperature Induced LTI-16
125	-	1-1,243	Xylem specific receptor-like kinase
126	-	1-1,047	Root specific
127	-	1-3,552	Elongation Factor 1-alpha

In one embodiment, the present invention provides polynucleotide sequences isolated from *Pinus radiata* and *Eucalyptus grandis* that encode a ubiquitin polypeptide. The full-length sequence of the ubiquitin polynucleotide isolated from *Pinus radiata* is provided in SEQ ID NO: 1, with the sequence of the promoter region including an intron being provided

in SEQ ID NO: 2 and the sequence of the promoter region excluding the intron being provided in SEQ ID NO: 3. The sequence of the ubiquitin polynucleotide isolated from *Eucalyptus grandis* is provided in SEQ ID NO: 34. In a related embodiment, the present invention provides isolated polypeptides encoded by the isolated polynucleotides of SEQ ID NO: 1 and 34, including polypeptides comprising the sequences of SEQ ID NO: 80 and 67.

The term "polynucleotide(s)," as used herein, means a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases and includes DNA and corresponding RNA molecules, including HnRNA and mRNA molecules, both sense and anti-sense strands, and comprehends cDNA, genomic DNA and recombinant DNA, as well as wholly or partially synthesized polynucleotides. An HnRNA molecule contains introns and corresponds to a DNA molecule in a generally one-to-one manner. An mRNA molecule corresponds to an HnRNA and DNA molecule from which the introns have been excised. A polynucleotide may consist of an entire gene, or any portion thereof. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all such operable anti-sense fragments. Anti-sense polynucleotides and techniques involving anti-sense polynucleotides are well known in the art and are described, for example, in Robinson-Benion *et al.* "Antisense techniques," *Methods in Enzymol.* 254(23):363-375, 1995; and Kawasaki *et al.*, in *Artific. Organs* 20(8):836-848, 1996.

All of the polynucleotides and polypeptides described herein are isolated and purified, as those terms are commonly used in the art. Preferably, the polypeptides and polynucleotides are at least about 80% pure, more preferably at least about 90% pure, and most preferably at least about 99% pure.

The definition of the terms "complement", "reverse complement" and "reverse sequence", as used herein, is best illustrated by the following example. For the sequence 5' AGGACC 3', the complement, reverse complement and reverse sequence are as follows:

Complement	3' TCCTGG 5'
Reverse complement	3' GGTCCT 5'
Reverse sequence	5' CCAGGA 3'

Some of the polynucleotides of the present invention are "partial" sequences, in that they do not represent a full-length gene encoding a full-length polypeptide. Such partial sequences may be extended by analyzing and sequencing various DNA libraries using primers and/or probes and well known hybridization and/or PCR techniques. Partial

sequences may be extended until an open reading frame encoding a polypeptide, a full-length polynucleotide and/or gene capable of expressing a polypeptide, or another useful portion of the genome is identified. Such extended sequences, including full-length polynucleotides and genes, are described as "corresponding to" a sequence identified as one of the sequences of SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, or a variant thereof, or a portion of one of the sequences of SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, or a variant thereof, when the extended polynucleotide comprises an identified sequence or its variant, or an identified contiguous portion (*x*-mer) of one of the sequences of SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, or a variant thereof. Such extended polynucleotides may have a length of from about 50 to about 4,000 nucleic acids or base pairs, and preferably have a length of less than about 4,000 nucleic acids or base pairs, more preferably yet a length of less than about 3,000 nucleic acids or base pairs, more preferably yet a length of less than about 2,000 nucleic acids or base pairs. Under some circumstances, extended polynucleotides of the present invention may have a length of less than about 1,800 nucleic acids or base pairs, preferably less than about 1,600 nucleic acids or base pairs, more preferably less than about 1,400 nucleic acids or base pairs, more preferably yet less than about 1,200 nucleic acids or base pairs, and most preferably less than about 1,000 nucleic acids or base pairs.

Similarly, RNA sequences, reverse sequences, complementary sequences, antisense sequences, and the like, corresponding to the polynucleotides of the present invention, may be routinely ascertained and obtained using the cDNA sequences identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127.

The polynucleotides identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, and their extensions, may contain open reading frames ("ORFs") or partial open reading frames encoding polypeptides. Additionally, open reading frames encoding polypeptides may be identified in extended or full length sequences corresponding to the sequences set out as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127. Open reading frames may be identified using techniques that are well known in the art. These techniques include, for example, analysis for the location of known start and stop codons, most likely reading frame identification based on codon frequencies, etc. Suitable tools and software for ORF analysis include, for example, "GeneWise", available from The Sanger Center, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, United Kingdom; "Diogenes", available from Computational Biology Centers, University of Minnesota, Academic Health Center, UMHG Box 43, Minneapolis MN 55455 and "GRAIL", available from the Informatics

Group, Oak Ridge National Laboratories, Oak Ridge, Tennessee TN. Open reading frames and portions of open reading frames may be identified in the polynucleotides of the present invention. Once a partial open reading frame is identified, the polynucleotide may be extended in the area of the partial open reading frame using techniques that are well known in the art until the polynucleotide for the full open reading frame is identified. Thus, open reading frames encoding polypeptides may be identified using the polynucleotides of the present invention.

Once open reading frames are identified in the polynucleotides of the present invention, the open reading frames may be isolated and/or synthesized. Expressible genetic constructs comprising the open reading frames and suitable promoters, initiators, terminators, etc., which are well known in the art, may then be constructed. Such genetic constructs may be introduced into a host cell to express the polypeptide encoded by the open reading frame. Suitable host cells may include various prokaryotic and eukaryotic cells, including plant cells, mammalian cells, bacterial cells, algae and the like.

Polypeptides encoded by the polynucleotides of the present invention may be expressed and used in various assays to determine their biological activity. Such polypeptides may be used to raise antibodies, to isolate corresponding interacting proteins or other compounds, and to quantitatively determine levels of interacting proteins or other compounds.

The term "polypeptide", as used herein, encompasses amino acid chains of any length including full length proteins, wherein amino acid residues are linked by covalent peptide bonds. Polypeptides of the present invention may be isolated and purified natural products, or may be produced partially or wholly using recombinant techniques. The term "polypeptide encoded by a polynucleotide" as used herein, includes polypeptides encoded by a nucleotide sequence which includes the partial isolated DNA sequences of the present invention.

In a related aspect, polypeptides are provided that comprise at least a functional portion of a polypeptide having a sequence selected from the group consisting of sequences provided in SEQ ID NO: 63-80, 87 and 130, and variants thereof. As used herein, the "functional portion" of a polypeptide is that portion which contains the active site essential for affecting the function of the polypeptide, for example, the portion of the molecule that is capable of binding one or more reactants. The active site may be made up of separate portions present on one or more polypeptide chains and will generally exhibit high binding affinity.

Functional portions of a polypeptide may be identified by first preparing fragments of the polypeptide by either chemical or enzymatic digestion of the polypeptide, or by mutation analysis of the polynucleotide that encodes the polypeptide and subsequent expression of the resulting mutant polypeptides. The polypeptide fragments or mutant polypeptides are then tested to determine which portions retain biological activity, using, for example, the representative assays provided below. A functional portion comprising an active site may be made up of separate portions present on one or more polypeptide chains and generally exhibits high substrate specificity.

Portions and other variants of the inventive polypeptides may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, wherein amino acids are sequentially added to a growing amino acid chain. (Merrifield, *J. Am. Chem. Soc.* 85: 2149-2154, 1963). Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer / Applied Biosystems, Inc. (Foster City, California), and may be operated according to the manufacturer's instructions. Variants of a native polypeptide may be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (Kunkel, *Proc. Natl. Acad. Sci. USA* 82: 488-492, 1985). Sections of DNA sequences may also be removed using standard techniques to permit preparation of truncated polypeptides.

As used herein, the term "variant" comprehends nucleotide or amino acid sequences different from the specifically identified sequences, wherein one or more nucleotides or amino acid residues is deleted, substituted, or added. Variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant sequences (polynucleotide or polypeptide) preferably exhibit at least 50%, more preferably at least 75%, and most preferably at least 90% identity to a sequence of the present invention. The percentage identity is determined by aligning the two sequences to be compared as described below, determining the number of identical residues in the aligned portion, dividing that number by the total number of residues in the inventive (queried) sequence, and multiplying the result by 100.

Polynucleotide and polypeptide sequences may be aligned, and percentage of identical residues in a specified region may be determined against other polynucleotide and polypeptide sequences, using computer algorithms that are publicly available. Two exemplary algorithms for aligning and identifying the similarity of polynucleotide sequences are the BLASTN and FASTA algorithms. Polynucleotides may also be analyzed using the BLASTX algorithm, which compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database. The similarity of polypeptide sequences may be examined using the BLASTP algorithm. The BLASTN algorithm Version 2.0.4 [Feb-24-1998], Version 2.0.6 [Sept-16-1998] and Version 2.0.11 [Jan-20-2000], set to the default parameters described in the documentation and distributed with the algorithm, are preferred for use in the determination of polynucleotide variants according to the present invention. The BLASTP algorithm, is preferred for use in the determination of polypeptide variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN, BLASTP, and BLASTX, is described in the publication of Altschul, *et al.*, "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," *Nucleic Acids Res.* 25: 3389-3402, 1997. The BLASTN software is available on the NCBI anonymous FTP server (ftp://ncbi.nlm.nih.gov) under /blast/executables/ and is available from the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894 USA.

The FASTA software package is available from the University of Virginia (University of Virginia, PO Box 9025, Charlottesville, VA 22906-9025). Version 2.0u4, February 1996, set to the default parameters described in the documentation and distributed with the algorithm, may be used in the determination of variants according to the present invention. The use of the FASTA algorithm is described in Pearson and Lipman, "Improved Tools for Biological Sequence Analysis," *Proc. Natl. Acad. Sci. USA* 85: 2444-2448, 1988; and Pearson, "Rapid and Sensitive Sequence Comparison with FASTP and FASTA," *Methods in Enzymol.* 183: 63-98, 1990.

The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to the E values and percentage identity for polynucleotide sequences: Unix running command: blastall -p blastn -d embldb -e 10 –G0 -E0 -r 1 -v 30 -b 30 -i queryseq –o results; the parameters are: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes

default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -r Reward for a nucleotide match (BLASTN only) [Integer]; -v Number of one-line descriptions (V) [Integer]; -b Number of alignments to show (B) [Integer]; -i Query File [File In]; and -o BLAST report Output File [File Out] Optional.

The following running parameters are preferred for determination of alignments and similarities using BLASTP that contribute to the E values and percentage identity of polypeptide sequences: blastall –p blastp –d swissprotdb –e 10 -G 0 -E 0 –v 30 –b 30 –i queryseq –o results; the parameters are: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -v Number of one-line descriptions (v) [Integer]; -b Number of alignments to show (b) [Integer]; -I Query File [File In]; -o BLAST report Output File [File Out] Optional.

The "hits" to one or more database sequences by a queried sequence produced by BLASTN, FASTA, BLASTP or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

The BLASTN, FASTA and BLASTP algorithms also produce "Expect" values for alignments. The Expect value (E) indicates the number of hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database, such as the preferred EMBL database, indicates true similarity. For example, an E value of 0.1 assigned to a polynucleotide hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion of the sequence with a similar score simply by chance. By this criterion, the aligned and matched portions of the polynucleotide sequences then have a probability of 90% of being the same. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN or FASTA algorithm.

According to one embodiment, "variant" polynucleotides and polypeptides, with reference to each of the polynucleotides and polypeptides of the present invention, preferably comprise sequences having the same number or fewer nucleic or amino acids than each of the polynucleotides or polypeptides of the present invention and producing an E value of 0.01 or

less when compared to the polynucleotide or polypeptide of the present invention. That is, a variant polynucleotide or polypeptide is any sequence that has at least a 99% probability of being the same as the polynucleotide or polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTN, FASTA, or BLASTP algorithms set at parameters described above. According to a preferred embodiment, a variant polynucleotide is a sequence having the same number or fewer nucleic acids than a polynucleotide of the present invention that has at least a 99% probability of being the same as the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or FASTA algorithms set at parameters described above. Similarly, according to a preferred embodiment, a variant polypeptide is a sequence having the same number or fewer amino acids than a polypeptide of the present invention that has at least a 99% probability of being the same as a polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTP algorithm set at the parameters described above.

Alternatively, variant polynucleotides of the present invention hybridize to the polynucleotide sequences recited in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, or complements, reverse sequences, or reverse complements of those sequences under stringent conditions. As used herein, "stringent conditions" refers to prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65° C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65°C.

The present invention also encompasses polynucleotides that differ from the disclosed sequences but that, as a consequence of the discrepancy of the genetic code, encode a polypeptide having similar activity to a polypeptide encoded by a polynucleotide of the present invention. Thus, polynucleotides comprising sequences that differ from the polynucleotide sequences recited in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, or complements, reverse sequences, or reverse complements thereof, as a result of conservative substitutions are contemplated by and encompassed within the present invention. Additionally, polynucleotides comprising sequences that differ from the polynucleotide sequences recited in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, or complements, reverse complements or reverse sequences thereof, as a result of deletions and/or insertions totaling less than 10% of the total sequence length are also contemplated by and encompassed within the present invention. Similarly, polypeptides comprising sequences that differ from the polypeptide sequences recited in SEQ ID NO: 63-80, 87 and 130, as a result of amino

acid substitutions, insertions, and/or deletions totaling less than 10% of the total sequence length are contemplated by an encompassed within the present invention. In certain embodiments, variants of the inventive polypeptides and polynucleotides possess biological activities that are the same or similar to those of the inventive polypeptides or polynucleotides. Such variant polynucleotides function as promoter sequences and are thus capable of modifying gene expression in a plant.

The polynucleotides of the present invention may be isolated from various libraries, or may be synthesized using techniques that are well known in the art. The polynucleotides may be synthesized, for example, using automated oligonucleotide synthesizers (*e.g.*, Beckman Oligo 1000M DNA Synthesizer) to obtain polynucleotide segments of up to 50 or more nucleic acids. A plurality of such polynucleotide segments may then be ligated using standard DNA manipulation techniques that are well known in the art of molecular biology. One conventional and exemplary polynucleotide synthesis technique involves synthesis of a single stranded polynucleotide segment having, for example, 80 nucleic acids, and hybridizing that segment to a synthesized complementary 85 nucleic acid segment to produce a 5-nucleotide overhang. The next segment may then be synthesized in a similar fashion, with a 5-nucleotide overhang on the opposite strand. The "sticky" ends ensure proper ligation when the two portions are hybridized. In this way, a complete polynucleotide of the present invention may be synthesized entirely *in vitro*.

Polynucleotides of the present invention also comprehend polynucleotides comprising at least a specified number of contiguous residues (*x*-mers) of any of the polynucleotides identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, complements, reverse sequences, and reverse complements of such sequences, and their variants. Similarly, polypeptides of the present invention comprehend polypeptides comprising at least a specified number of contiguous residues (*x*-mers) of any of the polypeptides identified as SEQ ID NO: 63-80, 87 and 130, and their variants. As used herein, the term "*x*-mer," with reference to a specific value of "*x*," refers to a sequence comprising at least a specified number ("*x*") of contiguous residues of any of the polynucleotides identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, or the polypeptides identified as SEQ ID NO: 63-80, 87 and 130. According to preferred embodiments, the value of *x* is preferably at least 20, more preferably at least 40, more preferably yet at least 60, and most preferably at least 80. Thus, polynucleotides and polypeptides of the present invention comprise a 20-mer, a 40-mer, a 60-mer, an 80-mer, a 100-mer, a 120-mer, a 150-mer, a 180-mer, a 220-mer, a 250-mer, a 300-

mer, 400-mer, 500-mer or 600-mer of a polynucleotide or polypeptide identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, and variants thereof.

As noted above, the inventive polynucleotide promoter sequences may be employed in genetic constructs to drive transcription and/or expression of a polynucleotide of interest. The polynucleotide of interest may be either endogenous or heterologous to an organism, for example a plant, to be transformed. The inventive genetic constructs may thus be employed to modulate levels of transcription and/or expression of a polynucleotide, for example gene, that is present in the wild-type plant, or may be employed to provide transcription and/or expression of a DNA sequence that is not found in the wild-type plant.

In certain embodiments, the polynucleotide of interest comprises an open reading frame that encodes a target polypeptide. The open reading frame is inserted in the genetic construct in either a sense or antisense orientation, such that transformation of a target plant with the genetic construct will lead to a change in the amount of polypeptide compared to the wild-type plant. Transformation with a genetic construct comprising an open reading frame in a sense orientation will generally result in over-expression of the selected polypeptide, while transformation with a genetic construct comprising an open reading frame in an antisense orientation will generally result in reduced expression of the selected polypeptide. A population of plants transformed with a genetic construct comprising an open reading frame in either a sense or antisense orientation may be screened for increased or reduced expression of the polypeptide in question using techniques well known to those of skill in the art, and plants having the desired phenotypes may thus be isolated.

Alternatively, expression of a target polypeptide may be inhibited by inserting a portion of the open reading frame, in either sense or antisense orientation, in the genetic construct. Such portions need not be full-length but preferably comprise at least 25 and more preferably at least 50 residues of the open reading frame. A much longer portion or even the full length DNA corresponding to the complete open reading frame may be employed. The portion of the open reading frame does not need to be precisely the same as the endogenous sequence, provided that there is sufficient sequence similarity to achieve inhibition of the target gene. Thus a sequence derived from one species may be used to inhibit expression of a gene in a different species.

In further embodiments, the inventive genetic constructs comprise a polynucleotide including an untranslated, or non-coding, region of a gene coding for a target polypeptide, or a polynucleotide complementary to such an untranslated region. Examples of untranslated

regions which may be usefully employed in such constructs include introns and 5'-untranslated leader sequences. Transformation of a target plant with such a genetic construct may lead to a reduction in the amount of the polypeptide expressed in the plant by the process of cosuppression, in a manner similar to that discussed, for example, by Napoli *et al.*, *Plant Cell* 2:279-290, 1990 and de Carvalho Niebel *et al.*, *Plant Cell* 7:347-358, 1995.

Alternatively, regulation of polypeptide expression can be achieved by inserting appropriate sequences or subsequences (e.g. DNA or RNA) in ribozyme constructs (McIntyre and Manners, *Transgenic Res.* 5(4):257-262, 1996). Ribozymes are synthetic RNA molecules that comprise a hybridizing region complementary to two regions, each of which comprises at least 5 contiguous nucleotides in a mRNA molecule encoded by one of the inventive polynucleotides. Ribozymes possess highly specific endonuclease activity, which autocatalytically cleaves the mRNA.

The polynucleotide of interest, such as a coding sequence, is operably linked to a polynucleotide promoter sequence of the present invention such that a host cell is able to transcribe an RNA from the promoter sequence linked to the polynucleotide of interest. The polynucleotide promoter sequence is generally positioned at the 5' end of the polynucleotide to be transcribed. Use of a constitutive promoter, such as the *Pinus radiata* ubiquitin polynucleotide promoter sequence of SEQ ID NO: 2 and 3' or the *Eucalyptus grandis* ubiquitin polynucleotide promoter sequence contained within SEQ ID NO: 34, will affect transcription of the polynucleotide of interest in all parts of the transformed plant. Use of a tissue specific promoter, such as the leaf-specific promoters of SEQ ID NO: 9-11, the root-specific promoters of SEQ ID NO: 13 and 14, the flower-specific promoters of SEQ ID NO: 29-33, 59 and 89-90, the pollen-specific promoters of SEQ ID NO: 49-55 and 94, the bud-specific promoter of SEQ ID NO: 40 or the meristem-specific promoter of SEQ ID NO: 45, will result in production of the desired sense or antisense RNA only in the tissue of interest. Temporally regulated promoters, such as the xylogenesis-specific promoters of SEQ ID NO: 5, 41-44 and 92, can be employed to effect modulation of the rate of DNA transcription at a specific time during development of a transformed plant. With genetic constructs employing inducible gene promoter sequences, the rate of DNA transcription can be modulated by external stimuli, such as light, heat, anaerobic stress, alteration in nutrient conditions and the like.

The inventive genetic constructs further comprise a gene termination sequence which is located 3' to the polynucleotide of interest. A variety of gene termination sequences which

may be usefully employed in the genetic constructs of the present invention are well known in the art. One example of such a gene termination sequence is the 3' end of the *Agrobacterium tumefaciens* nopaline synthase gene. The gene termination sequence may be endogenous to the target plant or may be exogenous, provided the promoter is functional in the target plant. For example, the termination sequence may be from other plant species, plant viruses, bacterial plasmids and the like.

The genetic constructs of the present invention may also contain a selection marker that is effective in cells of the target organism, such as a plant, to allow for the detection of transformed cells containing the inventive construct. Such markers, which are well known in the art, typically confer resistance to one or more toxins. One example of such a marker is the NPTII gene whose expression results in resistance to kanamycin or hygromycin, antibiotics which are usually toxic to plant cells at a moderate concentration (Rogers *et al., in* Weissbach A and H, eds. *Methods for Plant Molecular Biology*, Academic Press Inc.: San Diego, CA, 1988). Transformed cells can thus be identified by their ability to grow in media containing the antibiotic in question. Alternatively, the presence of the desired construct in transformed cells can be determined by means of other techniques well known in the art, such as Southern and Western blots.

Techniques for operatively linking the components of the inventive genetic constructs are well known in the art and include the use of synthetic linkers containing one or more restriction endonuclease sites as described, for example, by Sambrook *et al.*, (*Molecular cloning: a laboratory manual*, CSHL Press: Cold Spring Harbor, NY, 1989). The genetic construct of the present invention may be linked to a vector having at least one replication system, for example *E. coli*, whereby after each manipulation, the resulting construct can be cloned and sequenced and the correctness of the manipulation determined.

The genetic constructs of the present invention may be used to transform a variety of target organisms including, but not limited to, plants. Plants which may be transformed using the inventive constructs include both monocotyledonous angiosperms (*e.g.*, grasses, corn, grains, oat, wheat and barley) and dicotyledonous angiosperms (*e.g.*, *Arabidopsis*, tobacco, legumes, alfalfa, oaks, eucalyptus, maple), and Gymnosperms (*e.g.*, Scots pine; *see* Aronen, *Finnish Forest Res. Papers*, Vol. 595, 1996), white spruce (Ellis *et al.*, *Biotechnology* 11:84-89, 1993), and larch (Huang *et al.*, *In Vitro Cell* 27:201-207, 1991). In a preferred embodiment, the inventive genetic constructs are employed to transform woody plants, herein defined as a tree or shrub whose stem lives for a number of years and increases in diameter

each year by the addition of woody tissue. Preferably the target plant is selected from the group consisting of eucalyptus and pine species, most preferably from the group consisting of *Eucalyptus grandis* and *Pinus radiata*. Other species which may be usefully transformed with the genetic constructs of the present invention include, but are not limited to: pines such as *Pinus banksiana, Pinus brutia, Pinus caribaea, Pinus clausa, Pinus contorta, Pinus coulteri, Pinus echinata, Pinus eldarica, Pinus ellioti, Pinus jeffreyi, Pinus lambertiana, Pinus monticola, Pinus nigra, Pinus palustrus, Pinus pinaster, Pinus ponderosa, Pinus resinosa, Pinus rigida, Pinus serotina, Pinus strobus, Pinus sylvestris, Pinus taeda, Pinus virginiana*; other gymnosperms, such as *Abies amabilis, Abies balsamea, Abies concolor, Abies grandis, Abies lasiocarpa, Abies magnifica, Abies procera, Chamaecyparis lawsoniona, Chamaecyparis nootkatensis, Chamaecyparis thyoides, Huniperus virginiana, Larix decidua, Larix laricina, Larix leptolepis, Larix occidentalis, Larix siberica, Libocedrus decurrens, Picea abies, Picea engelmanni, Picea glauca, Picea mariana, Picea pungens, Picea rubens, Picea sitchensis, Pseudotsuga menziesii, Sequoia gigantea, Sequoia sempervirens, Taxodium distichum, Tsuga canadensis, Tsuga heterophylla, Tsuga mertensiana, Thuja occidentalis, Thuja plicata*; and Eucalypts, such as *Eucalyptus alba, Eucalyptus bancroftii, Eucalyptus botyroides, Eucalyptus bridgesiana, Eucalyptus calophylla, Eucalyptus camaldulensis, Eucalyptus citriodora, Eucalyptus cladocalyx, Eucalyptus coccifera, Eucalyptus curtisii, Eucalyptus dalrympleana, Eucalyptus deglupta, Eucalyptus delagatensis, Eucalyptus diversicolor, Eucalyptus dunnii, Eucalyptus ficifolia, Eucalyptus globulus, Eucalyptus gomphocephala, Eucalyptus gunnii, Eucalyptus henryi, Eucalyptus laevopinea, Eucalyptus macarthurii, Eucalyptus macrorhyncha, Eucalyptus maculata, Eucalyptus marginata, Eucalyptus megacarpa, Eucalyptus melliodora, Eucalyptus nicholii, Eucalyptus nitens, Eucalyptus nova-anglica, Eucalyptus obliqua, Eucalyptus obtusiflora, Eucalyptus oreades, Eucalyptus pauciflora, Eucalyptus polybractea, Eucalyptus regnans, Eucalyptus resinifera, Eucalyptus robusta, Eucalyptus rudis, Eucalyptus saligna, Eucalyptus sideroxylon, Eucalyptus stuartiana, Eucalyptus tereticornis, Eucalyptus torelliana, Eucalyptus urnigera, Eucalyptus urophylla, Eucalyptus viminalis, Eucalyptus viridis, Eucalyptus wandoo* and *Eucalyptus youmanni*; and hybrids of any of these species.

Techniques for stably incorporating genetic constructs into the genome of target plants are well known in the art and include *Agrobacterium tumefaciens* mediated introduction, electroporation, protoplast fusion, injection into reproductive organs, injection into immature embryos, high velocity projectile introduction and the like. The choice of

technique will depend upon the target plant to be transformed. For example, dicotyledonous plants and certain monocots and gymnosperms may be transformed by *Agrobacterium* Ti plasmid technology, as described, for example by Bevan, *Nucleic Acids Res.* 12:8711-8721, 1984. Targets for the introduction of the genetic constructs of the present invention include tissues, such as leaf tissue, dissociated cells, protoplasts, seeds, embryos, meristematic regions; cotyledons, hypocotyls, and the like. The preferred method for transforming eucalyptus and pine is a biolistic method using pollen (see, for example, Aronen, *Finnish Forest Res. Papers*, Vol. 595, 53pp, 1996) or easily regenerable embryonic tissues.

Once the cells are transformed, cells having the inventive genetic construct incorporated in their genome may be selected by means of a marker, such as the kanamycin resistance marker discussed above. Transgenic cells may then be cultured in an appropriate medium to regenerate whole plants, using techniques well known in the art. In the case of protoplasts, the cell wall is allowed to reform under appropriate osmotic conditions. In the case of seeds or embryos, an appropriate germination or callus initiation medium is employed. For explants, an appropriate regeneration medium is used. Regeneration of plants is well established for many species. For a review of regeneration of forest trees see Dunstan *et al.*, "Somatic embryogenesis in woody plants," *in* Thorpe TA, ed., *In Vitro Embryogenesis of Plants* (*Current Plant Science and Biotechnology in Agriculture Vol. 20*), Chapter 12, pp. 471-540, 1995. Specific protocols for the regeneration of spruce are discussed by Roberts *et al.*, "Somatic embryogenesis of spruce," *in* Redenbaugh K, ed., *Synseed: applications of synthetic seed to crop improvement*, CRC Press: Chapter 23, pp. 427-449, 1993). Transformed plants having the desired phenotype may be selected using techniques well known in the art. The resulting transformed plants may be reproduced sexually or asexually, using methods well known in the art, to give successive generations of transgenic plants.

As discussed above, the production of RNA in target cells can be controlled by choice of the promoter sequence, or by selecting the number of functional copies or the site of integration of the polynucleotides incorporated into the genome of the target host. A target organism may be transformed with more than one genetic construct of the present invention, thereby modulating the activity of more than gene. Similarly, a genetic construct may be assembled containing more than one open reading frame coding for a polypeptide of interest or more than one untranslated region of a gene coding for such a polypeptide.

The isolated polynucleotides of the present invention also have utility in genome mapping, in physical mapping, and in positional cloning of genes. As detailed below, the

polynucleotide sequences identified as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, and their variants, may be used to design oligonucleotide probes and primers. Oligonucleotide probes designed using the polynucleotides of the present invention may be used to detect the presence and examine the expression patterns of genes in any organism having sufficiently similar DNA and RNA sequences in their cells using techniques that are well known in the art, such as slot blot DNA hybridization techniques. Oligonucleotide primers designed using the polynucleotides of the present invention may be used for PCR amplifications. Oligonucleotide probes and primers designed using the polynucleotides of the present invention may also be used in connection with various microarray technologies, including the microarray technology of Affymetrix (Santa Clara, CA).

As used herein, the term "oligonucleotide" refers to a relatively short segment of a polynucleotide sequence, generally comprising between 6 and 60 nucleotides, and comprehends both probes for use in hybridization assays and primers for use in the amplification of DNA by polymerase chain reaction.

An oligonucleotide probe or primer is described as "corresponding to" a polynucleotide of the present invention, including one of the sequences set out as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, or a variant, if the oligonucleotide probe or primer, or its complement, is contained within one of the sequences set out as SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127, or a variant of one of the specified sequences. Oligonucleotide probes and primers of the present invention are substantially complementary to a polynucleotide disclosed herein.

Two single stranded sequences are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared, with the appropriate nucleotide insertions and/or deletions, pair with at least 80%, preferably at least 90% to 95% and more preferably at least 98% to 100% of the nucleotides of the other strand. Alternatively, substantial complementarity exists when a first DNA strand will selectively hybridize to a second DNA strand under stringent hybridization conditions. Stringent hybridization conditions for determining complementarity include salt conditions of less than about 1 M, more usually less than about 500 mM, and preferably less than about 200 mM. Hybridization temperatures can be as low as 5°C, but are generally greater than about 22°C, more preferably greater than about 30°C, and most preferably greater than about 37°C. Longer DNA fragments may require higher hybridization temperatures for specific hybridization. Since the stringency of hybridization may be affected by other factors such as probe composition,

presence of organic solvents and extent of base mismatching, the combination of parameters is more important than the absolute measure of any one alone.

In specific embodiments, the oligonucleotide probes and/or primers comprise at least about 6 contiguous residues, more preferably at least about 10 contiguous residues, and most preferably at least about 20 contiguous residues complementary to a polynucleotide sequence of the present invention. Probes and primers of the present invention may be from about 8 to 100 base pairs in length or, preferably from about 10 to 50 base pairs in length or, more preferably from about 15 to 40 base pairs in length. The probes can be easily selected using procedures well known in the art, taking into account DNA-DNA hybridization stringencies, annealing and melting temperatures, and potential for formation of loops and other factors, which are well known in the art.. Preferred techniques for designing PCR primers are disclosed in Dieffenbach, CW and Dyksler, GS. *PCR Primer: a laboratory manual*, CSHL Press: Cold Spring Harbor, NY, 1995. A software program suitable for designing probes, and especially for designing PCR primers, is available from Premier Biosoft International, 3786 Corina Way, Palo Alto, CA 94303-4504.

A plurality of oligonucleotide probes or primers corresponding to a polynucleotide of the present invention may be provided in a kit form. Such kits generally comprise multiple DNA or oligonucleotide probes, each probe being specific for a polynucleotide sequence. Kits of the present invention may comprise one or more probes or primers corresponding to a polynucleotide of the present invention, including a polynucleotide sequence identified in SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-120.

In one embodiment useful for high-throughput assays, the oligonucleotide probe kits of the present invention comprise multiple probes in an array format, wherein each probe is immobilized at a predefined, spatially addressable location on the surface of a solid substrate. Array formats which may be usefully employed in the present invention are disclosed, for example, in U.S. Patents No. 5,412,087 and 5,545,451; and PCT Publication No. WO 95/00450, the disclosures of which are hereby incorporated by reference.

The polynucleotides of the present invention may also be used to tag or identify an organism or reproductive material therefrom. Such tagging may be accomplished, for example, by stably introducing a non-disruptive non-functional heterologous polynucleotide identifier into an organism, the polynucleotide comprising one of the polynucleotides of the present invention.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

Isolation and Characterization of a Ubiquitin Gene Promoter from *Pinus radiata*

Pinus radiata cDNA expression libraries were constructed and screened as follows. mRNA was extracted from plant tissue using the protocol of Chang *et al.*, *Plant Molecular Biology Reporter* 11:113-116, 1993 with minor modifications. Specifically, samples were dissolved in CPC-RNAXB (100 mM Tris-Cl, pH 8,0; 25 mM EDTA; 2.0 M NaCl; 2%CTAB; 2% PVP and 0.05% Spermidine*3HCl) and extracted with chloroform:isoamyl alcohol, 24:1. mRNA was precipitated with ethanol and the total RNA preparate was purified using a Poly(A) Quik mRNA Isolation Kit (Stratagene, La Jolla, CA). A cDNA expression library was constructed from the purified mRNA by reverse transcriptase synthesis followed by insertion of the resulting cDNA clones in Lambda ZAP using a ZAP Express cDNA Synthesis Kit (Stratagene), according to the manufacturer's protocol. The resulting cDNAs were packaged using a Gigapack II Packaging Extract (Stratagene) employing 1 μl of sample DNA from the 5 μl ligation mix. Mass excision of the library was done using XL1-Blue MRF' cells and XLOLR cells (Stratagene) with ExAssist helper phage (Stratagene). The excised phagemids were diluted with NZY broth (Gibco BRL, Gaithersburg, MD) and plated out onto LB-kanamycin agar plates containing X-gal and isopropylthio-beta-galactoside (IPTG).

Of the colonies plated and picked for DNA miniprep, 99% contained an insert suitable for sequencing. Positive colonies were cultured in NZY broth with kanamycin and cDNA was purified by means of alkaline lysis and polyethylene glycol (PEG) precipitation. Agarose gel at 1% was used to screen sequencing templates for chromosomal contamination. Dye primer sequences were prepared using a Turbo Catalyst 800 machine (Perkin Elmer/Applied Biosystems Division, Foster City, CA) according to the manufacturer's protocol.

DNA sequence for positive clones was obtained using a Perkin Elmer/Applied Biosystems Division Prism 377 sequencer. cDNA clones were sequenced first from the 5' end and, in some cases, also from the 3' end. For some clones, internal sequence was obtained using subcloned fragments. Subcloning was performed using standard procedures of restriction mapping and subcloning to pBluescript II SK+ vector.

As described below, one of the most abundant sequences identified was a ubiquitin gene, hereinafter referred to as the "Super-Ubiquitin or SU" gene.

Isolation of cDNA clones containing the ubiquitin gene

Sequences of cDNA clones with homology to the ubiquitin gene were obtained from high-throughput cDNA sequencing as described above. Sequences from several independent clones were assembled in a contig and a consensus sequence was generated from overlapping clones. The determined nucleotide sequence of the isolated Super Ubiquitin clone, comprising the promoter region (including an intron), coding region and 3' untranslated region (UTR) is provided in SEQ ID NO: 1. The 5' UTR is represented by residues 1 to 2,064, the intron by residues 1,196 to 2,033, and the coding region of the gene, which contains three direct repeats, by residues 2,065 to 2,751. The 3' UTR is 328 residues long (residues 2,755 to 3,083). The nucleotide sequence of the Super Ubiquitin promoter region only, including the intron, is given in SEQ ID NO: 2. The nucleotide sequence of the Super Ubiquitin promoter region only, excluding the intron, is given in SEQ ID NO: 3. The predicted amino acid sequence for the *Pinus radiata* Super Ubiquitin is provided in SEQ ID NO: 80.

Ubiquitin proteins function as part of a protein degradation pathway, in which they covalently attach to proteins, thereby targeting them for degradation (for a review, see Belknap and Garbarino, *Trends in Plant Sciences* 1:331-335, 1996). The protein is produced from a precursor polypeptide, encoded by a single mRNA. The Super Ubiquitin mRNA contains three copies of the ubiquitin monomer.

Cloning of the Super Ubiquitin Promoter

Fragments of the Super Ubiquitin promoter were cloned by two different PCR-based approaches.

<u>Method 1: Long Distance Gene Walking PCR</u>

Using "Long Distance Gene Walking" PCR (Min and Powell, *Biotechniques* 24:398-400, 1998), a 2 kb fragment was obtained that contained the entire coding region of the ubiquitin gene, a 900 bp intron in the 5' UTR and approximately 100 bp of the promoter.

To generate this fragment, 2 nested primers were designed from the 3' UTR of the Super Ubiquitin cDNA sequence isolated from pine. Generally, the 5' UTR is used for

primer design to amplify upstream sequence. However, the available 5' UTR of Super Ubiquitin was very short, and two initial primers derived from this region failed to amplify any fragments. Therefore, the primers of SEQ ID NO: 15 and 16 were designed from the 3' UTR.

The method involved an initial, linear PCR step with pine genomic DNA as template using the primer of SEQ ID NO: 15, and subsequent C-tailing of the single stranded DNA product using terminal transferase. The second PCR-step used these fragments as template for amplification with the primer of SEQ ID NO: 16 and primer AP of SEQ ID NO: 17. The AP primer was designed to bind to the polyC tail generated by the terminal transferase. Both primers (SEQ ID NO: 16 and 17) contained a 5'-*Not*I restriction site for the cloning of products into the *Not*I site of a suitable vector. The final PCR product contained fragments of different sizes. These fragments were separated by electrophoresis and the largest were purified from the gel, digested with restriction endonuclease *Not*I and cloned in the *Not*I site of expression vector pBK-CMV (Stratagene, La Jolla, CA). The largest of these clones contained the complete coding region of the gene (no introns were found in the coding sequence) and a 5' UTR which contained a 900 bp intron.

Method 2: "Genome Walker" kit

The Super Ubiquitin gene promoter was cloned using a "Genome Walker" kit (Clontech, Palo Alto, CA). This is also a PCR-based method, which requires two PCR primers to be constructed, one of which must be gene-specific. Although the ubiquitin coding region is highly conserved, the 5' UTR from different ubiquitin genes is not conserved and could therefore be used to design a gene-specific primer. A 2.2 kb fragment was amplified and subcloned in pGEM-T-easy (Promega, Madison, WI). Analysis by PCR and DNA sequencing showed that the clone contained 5' UTR sequence of the Super Ubiquitin gene, including the 900 bp intron and approximately 1 kb of putative promoter region. An intron in the 5' UTR is a common feature of plant polyubiquitin genes and may be involved in determining gene expression levels.

The gene specific primers used for these PCR reactions are provided in SEQ ID NO: 18 and 19.

Expression of Super Ubiquitin

Using primers derived from the gene-specific 5' and 3' UTR sequences, expression levels of Super Ubiquitin in different plant tissues was examined by means of RT-PCR. Super Ubiquitin was found to be expressed in all plant tissues examined, including branch phloem and xylem, feeder roots, fertilized cones, needles, one year old cones, pollen sacs, pollinated cones, root xylem, shoot buds, structural roots, trunk phloem and trunk. Expression of Super Ubiquitin in plant tissues was also demonstrated in a Northern blot assay using a PCR probe prepared from the 5'UTR.

Functional analysis of the Super Ubiquitin Promoter

To test the function of the Super Ubiquitin promoter in plants, *Arabidopsis thaliana* was transformed with constructs containing the reporter gene for Green Fluorescent Protein (GFP) operably linked to either the Super Ubiquitin promoter of SEQ ID NO: 2 or SEQ ID NO: 3 (i.e., either with or without the intron). Constructs lacking a promoter were used as a negative control, with a plant T-DNA vector carrying a CaMV 35S promoter cloned in front of GFP being used as a positive control. The constructs were introduced into *Arabidopsis* via *Agrobacterium*-mediated transformation.

All the plant culture media were according to the protocol of Valvekens and Van Montagu, *Proc. Natl. Acad. Sci. USA* 85:5536-5540, 1988 with minor modifications. For root transformation, sterilized seeds were placed in a line on the surface of germination medium, the plates were placed on their sides to facilitate root harvesting, and the seeds were grown for two weeks at 24°C with a 16 h photoperiod.

Expression of the constructs was measured by determining expression levels of the reporter gene for Green Fluorescent Protein (GFP). Preliminary GFP expression (transient) was detected in early transgenic roots during T-DNA transfer. Transgenic roots that developed green callus, growing on shoot-inducing medium containing 50 μg/ml Kanamycin and 100 μg/ml Timentin, were further tested for GFP expression. After several weeks of stringent selection on Kanamycin medium, several independent transgenic *Arabidopsis* lines were engineered and tested for GFP expression.

Expression was seen both with the Super Ubiquitin promoter including intron and the Super Ubiquitin promoter without the intron. However, preliminary results indicated that the levels of expression obtained with the Super Ubiquitin intron-less promoter construct were

significantly higher than those seen with the promoter including intron, suggesting that the intron may contain a repressor. The sequence of the intron is provided in SEQ ID NO: 21.

EXAMPLE 2

Isolation of a CDC Promoter from *Pinus radiata*

Plant polynucleotide sequences homologous to the Cell Division Control (CDC) protein gene were isolated from a *Pinus radiata* cDNA expression library as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, 5'UTR sequence containing the putative promoter of the *P. radiata* CDC gene was isolated from genomic DNA. The determined nucleotide sequence is given in SEQ ID NO: 4.

EXAMPLE 3

Isolation of a Xylogenesis-Specific Promoter from *Pinus radiata*

Plant polynucleotide sequences specific for plant xylogenesis were isolated from *Pinus radiata* cDNA expression libraries prepared from xylem, essentially as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, sequences containing putative *Pinus radiata* xylogenesis-specific promoters were isolated from genomic DNA. The determined nucleotide sequences are provided in SEQ ID NO: 5 and 41-44. An extended cDNA sequence for the clone of SEQ ID NO: 41-44 is provided in SEQ ID NO: 92.

EXAMPLE 4

Isolation of a 4-Coumarate-CoA Ligase Promoter from *Pinus radiata*

Plant polynucleotide sequences homologous to the 4-Coumarate-CoA Ligase (4CL) gene were isolated from a *Pinus radiata* cDNA expression library as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, sequences containing the putative promoter of the *P. radiata* 4CL gene was isolated from genomic DNA. The determined nucleotide sequence is given in SEQ ID NO: 6.

Genetic constructs comprising the reporter gene for Green Fluorescent Protein (GFP) or GUS reporter genes operably linked to the promoter of SEQ ID NO: 6 were prepared and used to transform *Arabidopsis thaliana* plants.

EXAMPLE 5

Isolation of a Cellulose Synthase Promoter from *Eucalyptus grandis*

Plant polynucleotide sequences homologous to the cellulose synthase gene were isolated from a *Eucalyptus grandis* cDNA expression library essentially as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, 5'UTR sequences containing the putative promoter of the *E. grandis* cellulose synthase gene were isolated from genomic DNA. Independent PCR experiments using different DNA bands as templates yielded two sequences which contained a number of base differences. One band was 750 bp in length and the nucleotide sequence of this band is given in SEQ ID NO: 7. The other band was 3 kb in length. The sequence of the 3' end of this band corresponded to the sequence given in SEQ ID NO: 7, with a number of base pair differences. The sequence of this 3' end is given in SEQ ID NO: 8. The sequence of the 5' end of this band is given in SEQ ID NO: 20.

EXAMPLE 6

Isolation of a Leaf-Specific Promoter from *Eucalyptus grandis*

Plant polynucleotide sequences specific for leaf were isolated from *Eucalyptus grandis* cDNA expression libraries prepared from leaf tissue, essentially as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, 5'UTR sequence containing a leaf-specific promoter of a novel *E. grandis* gene (of unknown function) was isolated from genomic DNA. Independent PCR experiments using different DNA bands as templates yielded three sequences which contained a number of base differences and deletions. The determined nucleotide sequences of the three PCR fragments are given in SEQ ID NO: 9-11.

EXAMPLE 7

Isolation of an O-Methyl Transferase Promoter from *Eucalyptus grandis*

Plant polynucleotide sequences homologous to an O-methyl transferase (OMT) gene were isolated from a *Eucalyptus grandis* cDNA expression library essentially as described in

Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, 5'UTR sequences containing the putative promoter of the *E. grandis* OMT gene was isolated from genomic DNA. The determined nucleotide sequence is given in SEQ ID NO: 12. This promoter sequence was extended by further sequencing. The extended cDNA sequences are given in SEQ ID NO: 60 and 113.

Genetic constructs comprising the reporter gene for Green Fluorescent Protein (GFP) operably linked to the promoter of SEQ ID NO: 12 were prepared and used to transform *Arabidopsis thaliana*.

EXAMPLE 8

Isolation of Root-Specific Promoters from *Pinus radiata*

Plant polynucleotide sequences homologous to the root-specific receptor-like kinase gene were isolated from a *Pinus radiata* cDNA expression library as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, 5'UTR sequence containing a putative *P. radiata* root-specific promoter was isolated from genomic DNA. Two independent PCR experiments yielded sequences that contained a number of base differences. The determined nucleotide sequences from the two experiments are given in SEQ ID NO: 13, 14, 110 and 111.

EXAMPLE 9

Isolation of an EF1-alpha Promoter from *Eucalyptus Grandis*

Plant polynucleotide sequences homologous to the *Eucalyptus* Elongation Factor-alpha (EF1-alpha) gene were isolated from a *Eucalyptus grandis* cDNA expression library and used to screen a *Eucalyptus grandis* genomic DNA library as follows.

The *Eucalyptus grandis* genomic DNA library was constructed using genomic DNA extracted from *Eucalyptus nitens x grandis* plant tissue, according to the protocol of Doyle and Doyle, *Focus* 12:13-15, 1990, with minor modifications. Specifically, plant tissue was ground under liquid nitrogen and dissolved in 2X CTAB extraction buffer (2% CTAB, hexadecyltrimethylammonium bromide; 1.4 M NaCl, 20 mM EDTA pH 8.0, 100 mM Tris.HCl. pH 8.0, 1% polyvinylpyrollidone). After extraction with chloroform: isoamylalcohol (24:1), 10% CTAB was added to the aqueous layer and the

chloroform:isoamylalcohol extraction repeated. . Genomic DNA was precipitated with isopropanol.

The resulting DNA was digested with restriction endonuclease *Sau*3A1 following standard procedures, extracted once with phenol:chloroform:isoamylalcohol (25:24:1) and ethanol precipitated. The digested fragments were separated on a sucrose density gradient using ultracentrifugation. Fractions containing fragments of 9-23 kb were pooled and ethanol precipitated. The resulting fragments were cloned into the lambda DASH II/*Bam*HI vector (Stratagene, La Jolla, CA) following the manufacturer's protocol and packaged using a Gigapack II Packaging Extract (Stratagene). The library was amplified once.

The library was screened with radiolabeled EST fragments isolated from a *Eucalyptus grandis* library (as described in Example 1), that showed homology to the *Eucalyptus* EF1-alpha gene. Phage lysates were prepared from positive plaques and genomic DNA was extracted.

From this genomic DNA, the 5'UTR region containing the putative promoter of the *Eucalyptus* EF1-alpha gene was obtained using the ELONGASE Amplification System (Gibco BRL). A 10 kb fragment was amplified and restriction mapped. The putative promoter region of the *Eucalyptus* elongation factor A (EF1-alpha) gene was identified on a 4kb fragment, which was subcloned into a pUC19 vector (Gibco BRL) containing an engineered *Not*I-site. The determined genomic DNA sequences of the isolated fragment containing the promoter region are provided in SEQ ID NO: 61 and 62, with the amino acid encoded by SEQ ID NO: 61 being provided in SEQ ID NO: 79. An extended sequence of the clone of SEQ ID NO: 61 is provided in SEQ ID NO: 127.

EXAMPLE 10

Isolation of Flower-Specific Promoters from *Eucalyptus grandis*

Plant polynucleotide sequences specific for flower-derived tissue were isolated from *Eucalyptus grandis* cDNA expression libraries prepared from flower tissue, essentially as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, several sequences, each containing a putative *Eucalyptus grandis* flower-specific promoter, were isolated from genomic DNA. The determined nucleotide sequences are given in SEQ ID NO: 29-33 and 59. An extended sequence of the clone of SEQ ID NO: 30-33 is provided in SEQ ID NO: 89. An extended sequence of the clone of SEQ ID NO: 29 is provided in SEQ ID NO: 90.

EXAMPLE 11

Isolation of Pollen-Specific Promoters from *Eucalyptus grandis* and *Pinus radiata*

Plant polynucleotide sequences specific for pollen were isolated from *Eucalyptus grandis* and *Pinus radiata* cDNA expression libraries prepared from pollen, essentially as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, several sequences, each containing a putative pollen-specific promoter, were isolated from genomic DNA. The determined nucleotide sequences isolated from *Pinus radiata* are given in SEQ ID NO: 49-53, with the predicted amino acid sequences encoded by SEQ ID NO: 51-53 being provided in SEQ ID NO: 73-75, respectively. An extended sequence for the clone of SEQ ID NO: 49 is provided in SEQ ID NO: 94.

EXAMPLE 12

Isolation of Bud-Specific and Meristem-Specific Promoter from *Pinus radiata*

Plant polynucleotide sequences specific for bud and meristem were isolated from *Pinus radiata* cDNA expression libraries prepared from bud and meristem, essentially as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, two sequences, one containing a putative bud-specific promoter and the other containing a putative meristem-specific promoter, were isolated from genomic DNA. The determined nucleotide sequences for these two promoters are given in SEQ ID NO: 40 and 45, respectively. The predicted amino acid sequences encoded by the DNA sequences of SEQ ID NO: 40 and 45 are provided in SEQ ID NO: 70 and 71, respectively.

EXAMPLE 13

Isolation of Promoters from *Eucalyptus grandis*

Plant polynucleotide sequences showing some homology to various known genes were isolated from *Eucalyptus grandis* cDNA expression libraries essentially as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, sequences containing the putative promoters for the following *E. grandis* genes were isolated from genomic DNA: auxin induced protein (SEQ ID NO: 26-28); carbonic anhydrase (SEQ ID NO: 36); isoflavone

reductase (SEQ ID NO: 37 and 38); pollen allergen (SEQ ID NO: 23-25); pollen coat protein (SEQ ID NO: 22), sucrose synthase (SEQ ID NO: 56-58); ubiquitin (SEQ ID NO: 34); glyceraldehyde-3-phosphate dehydrogenase (SEQ ID NO: 35 and 39); O-methyl transferase (OMT; SEQ ID NO: 60); macrophage migration inhibition factor from mammals (MIF; SEQ ID NO: 81-86); UDP glucose 6-dehydrogenase (SEQ ID NO: 103); laccase 1 (SEQ ID NO: 105, 106 and 116); arabinogalactan-like 1 (SEQ ID NO: 107); arabinogalactan-like 2 (SEQ ID NO: 108, 109); a hypothetical protein (SEQ ID NO: 104); constans (SEQ ID NO: 118); Flowering Promoting Factor 1 (FPF1; SEQ ID NO: 119); transcription factor DREB-1 (SEQ ID NO: 121); salt tolerance protein (SE QID NO: 123); xylem-specific histidine kinase-like (SEQ ID NO: 125) and root specific (SEQ ID NO: 126). The amino acid sequences encoded by the DNA sequences of SEQ ID NO: 22, 25, 26, 28, 34, 35, 36, 56, 57, 60, 86 and 124 are provided in SEQ ID NO: 63, 64, 65, 66, 67, 68, 69, 76, 77, 78, 87 and 130, respectively. Extended cDNA sequences for the clones of SEQ ID NO: 58, 35, 60, 103, 106 and 107 are provided in SEQ ID NO: 91, 93, 113 and 115-117, respectively.

EXAMPLE 14

Isolation of Promoters from *Pinus radiata*

Plant polynucleotide sequences showing some homology to various known genes were isolated from *Pinus radiata* cDNA expression libraries essentially as described in Example 1. Using the "Genome Walker" protocol described above and gene specific primers designed from these plant polynucleotide sequences, sequences containing the putative promoters for the following *Pinus radiata* genes were isolated from genomic DNA: senescence-like protein (SEQ ID NO: 46-48); nodulin homolog pollen specific (SEQ ID NO: 54 and 55); chalcone synthase (SEQ ID NO: 88); PrMALE1 (SEQ ID NO: 95, 96); UDP glucose glycosyltransferase (SEQ ID NO: 97); elogation factor 1 alpha (SEQ ID NO: 98, 99); S-adenosylmethionine synthase (SEQ ID NO: 100-102); *Pinus radiata* lipid transfer protein 2 (PrLTP2; SEQ ID NO: 112); *Pinus radiata* agamous protein (SEQ ID NO: 120); Drought Induced DI-19 (SEQ ID NO: 122) and low temperature induced protein LTI (SEQ ID NO 124). The amino acid sequences encoded by the polynucleotide sequences of SEQ ID NOS: 46 and 124 are provided in SEQ ID NOS: 72 and 130. An extended cDNA sequence for the clone of SEQ ID NO: 97 is provided in SEQ ID NO: 114.

EXAMPLE 15

Polynucleotide and Amino Acid Analysis

The determined cDNA sequences described above were compared to and aligned with known sequences in the EMBL database (as updated to October 2000). Specifically, the polynucleotides identified in SEQ ID NOS: 22-62 and 88-120 were compared to polynucleotides in the EMBL database using the BLASTN algorithm Version 2.0.6 [Sept-16-1998] and the polynucleotides identified in SEQ ID NOS: 121-127 were compared to polynucleotides in the EMBL database using the BLASTN algorithm Version 2.0.11 [Jan-20-2000] set to the following running parameters: Unix running command: blastall –p blastn –d embldb –e 10 –G0 –E0 –r1 –v30 –b30 –i queryseq –o results. Multiple alignments of redundant sequences were used to build up reliable consensus sequences. Based on similarity to known sequences from other plant or non-plant species, the isolated polynucleotides of the present invention identified as SEQ ID NOS: 22-62 and 88-127 were putatively identified as having the functions shown in Table 1, above.

The cDNA sequences of SEQ ID NO: 1-22, 23, 25-42, 45-49, 57-59, 62, 88-99, 101-112 and 114-127 were determined to have less than 40% identity to sequences in the EMBL database using the computer algorithm BLASTN, as described above. The cDNA sequences of SEQ ID NO: 56 and 113 were determined to have less than 60% identity to sequences in the EMBL database using BLASTN, as described above. The cDNA sequences of SEQ ID NO: 43, 52, 60 and 61 were determined to have less than 75% identity to sequences in the EMBL database using BLASTN, as described above. The cDNA sequences of SEQ ID NO: 24, 51 and 100 were determined to have less than 90% identity to sequences in the EMBL database using BLASTN, as described above.

EXAMPLE 16

Modification of a Reporter Gene under Control of the Superubiquitin Promoter

Six independent *Arabidopsis thaliana* transgenic lines were transformed with *Pinus radiata* superubiquitin promoter constructs to demonstrate the relative expression of a GUS reporter gene under control of different superubiquitin promoter constructs. The reporter constructs in the plasmid pBI-101 contained the GUS (β-D-glucuronidase) reporter gene in frame with the superubiquitin promoter with the intron (SEQ ID NO: 2), the superubiquitin

promoter without the intron (SEQ ID NO: 3), and the CaMV 35S promoter. A reporter gene construct without a promoter sequence was used as control.

Groups of six *Arabidopsis thaliana* plants were transformed with the reporter constructs described above, using *Agrobacterium tumefaciens* transformation protocols. *A. tumefaciens* was transformed with 100 ng of the plasmid DNA according to standard techniques, as described, for example, by Bevan (*Nucleic Acids Res.* 12:8711-8721, 1984). Fresh plant material was collected from each plant, protein extracted from the whole plant, and the protein concentration determined (Bradford, *Anal. Biochem.* 72:248-254, 1976). The protein samples were diluted with carrier bovine serum albumin to 100 ng protein to maintain readings on the fluorimeter in the linear part of the standard curve using 4-methyl-umbelliferone (MU). GUS activity was quantified by fluorimetric analysis, using a Victor^2 1420 multi-label counter (Wallac, Turku, Finland) as described by Jefferson (*Plant Mol. Biol. Rep.* 5:387-405, 1987). As shown in Fig. 1, the construct containing the superubiquitin promoter without the intron showed seven times more GUS activity than the CaMV 35S promoter and the construct containing the superubiquitin promoter with the intron showed sixty two times more GUS activity than the CaMV 35S promoter. No activity was detected for the promoter-less control construct.

EXAMPLE 17

Determination of the Activity of Superubiquitin Promoter Constructs in Tobacco Plant Protoplasts

Isolation of protoplasts

Protoplasts were isolated from sterile tobacco (*Nicotiana tabacum*) leaf tissue and transformed with superubiquitin promoter constructs. Mesophyll protoplasts were prepared according to the method of Bilang *et al.*, *Plant Molecular Biology Manual* A1:1-16, 1994. A number of fully expanded leaves were removed from sterile wild type tobacco plants, sliced perpendicular to the midrib and submerged in a digestion enzyme solution containing 1.2% cellulase and 0.4% pectinase (Sigma, St. Louis MO). The leaves were left to incubate in the dark without agitation at 26°C for approximately 18 hours. The leaf strips were then gently agitated for 30 min to release the protoplasts. Protoplasts were further purified by filtration through 100 µm nylon mesh. One ml of W5 solution (154 mM $MgCl_2$, 125 mM $CaCl_2$, 5 mM KCl, 5 mM glucose, pH 5.8 - 6) was carefully layered on top of the filtrate and

centrifuged at 80 x g for 10 min. The live protoplast layer was removed with a wide bore pipette, washed twice with 10 ml W5 solution using centrifugation at 70 x g for 5 min, with final resuspension in 5 ml W5 solution. Protoplasts were counted in a hemocytometer and viability was determined under the microscope after staining with 5 mg/ml fluoroscein diacetate (FDA) in 100% acetone.

Transformation with promoter constructs

The isolated protoplasts were transformed with plasmid DNA using a polyethylene glycol protocol. After centrifugation of the purified protoplasts at 70 x g for 5 min, they were resuspended in MMM solution (15 mM $MgCl_2$, 0.1% w/v 2[N-morpholino]ethanesulfonic acid (MES), 0.5 M mannitol pH 5.8) to a density of 2 x 10^6 protoplasts/ml. Aliquots containing 5 x 10^5 protoplasts/ml in 250 µl were distributed to 15 ml tubes and mixed with 20 µg plasmid DNA. 250 µl polyethylene glycol-4000 (40%) was gently added and incubated for 5 minutes at room temperature. Ten ml W5 solution was slowly added, the protoplasts centrifuged at 70 x g for 5 min and finally resuspended in 2 ml K3 medium (Bilang *et al.*, *Plant Molecular Biology Manual* A1:1-16, 1994). The transformed protoplasts were incubated in the dark at 26°C for 24 hours before protein was extracted for reporter enzyme assays using 4-methyl-umbelliferyl-glucuronide (MUG).

Protein was extracted from the protoplasts using the following protocol. Transformed protoplast suspensions were centrifuged at 70 x g for 10 min, resuspended in 50 µl extraction buffer (Jefferson, *Plant Mol. Biol. Rep.* 5:387-405, 1987) and vigorously mixed using a vortex. The homogenate was cleared by centrifugation at 4,300 rpm for 5 min, the supernatant removed and used for protein assays (Bradford, *Anal. Biochem.* 72:248-254, 1976).

The results shown in Fig. 2 demonstrate the promoter activity of deletion constructs of the superubiquitin promoter without the intron (SEQ ID NO: 3) and the superubiquitin promoter with the intron (SEQ ID NO: 2) in tobacco plant protoplasts transformed as described above. The deletion constructs were made in plasmid pBI-101 that contained the GUS reporter gene, using Endonuclease III (Gibco BRL, Gaithersburg, MD) according to the manufacturer's protocols. The deletion constructs contained 1,103; 753; 573; 446; 368 and 195 bp of superubiquitin promoter sequence, respectively, upstream of the TATA sequence (bp numbers 1,104-1,110 of SEQ ID NO: 2). A control construct containing no sequence upstream of the TATA sequence was also made. These results show that the construct

containing the entire superubiquitin promoter with the intron had the highest MU activity in the protoplasts.

In Fig 3, the tobacco protoplasts were transformed with four different promoter constructs in plasmid pBI-101 containing the GUS reporter gene. These included the superubiquitin promoter without the intron (SEQ ID NO: 3), an elongation factor 1α promoter (SEQ ID NO: 99) and a 5-adenosylmethionine synthetase promoter (SEQ ID NO: 100). A promoterless control was included in the experiment, and is referred to in Fig. 3 as pBI-101.

EXAMPLE 18

Determination of the Activity of *P. radiata* Pollen-specific Promoter and *E. grandis* Pollen Specific Promoter Constructs in transformed *Arabidopsis thaliana* cv Columbia

Arabidopsis thaliana transgenic lines were transformed with *A. tumefaciens* containing constructs of the *P. radiata* pollen specific promoter (SEQ ID NO: 94) and *E. grandis* pollen specific promoter (SEQ ID NO: 22) to demonstrate the relative expression of a GUS reporter gene under control of these promoter constructs. The promoter sequences were cloned into plasmid pBI-101 containing a GUS reporter gene.

Agrobacterium tumefaciens transformation

Agrobacterium tumefaciens strain GV3101 was transformed with these constructs using electroporation. Electrocompetent *A. tumefaciens* cells were prepared according to the method of Walkerpeach and Velten, *Plant Mol. Biol. Man.* B1:1-19, 1994. Construct DNA (4 ng) was added to 40 µl competent *A. tumefaciens* GV3101 cells and electroporation was done using a BTX Electro Cell Manipulator 600 at the following settings: Mode: T 2.5kV Resistance high voltage (HV), Set Capacitance: C (not used in HV mode), Set Resistance: R R5 (129 Ohm), Set charging voltage: S 1.44kV, Desired field strength: 14.4kV/cm and Desired pulse strength: t 5.0 msec. 400 µl YEP liquid media (20g/l yeast, 20 g/l peptone and 10 g/l sodium chloride) was added to the cuvette and left to recover for one hour at room temperature. Transformed bacteria in YEP medium were spread out on solid YEP medium containing 50 mg/l kanamycin and 50 mg/l rifampicin and incubated at 29°C for two days to allow colony growth.

Confirmation of transformation of constructs into *A. tumefaciens*

To confirm that the constructs have been transformed into *A. tumefaciens*, DNA from the *A. tumefaciens* colonies from the YEP plates were isolated using standard protocols and amplified using the polymerase chain reaction (PCR) with primers designed from the pBI-101 vector sequence. The primer sequences are given in SEQ ID NOS: 128 and 129. PCR reactions were set up following standard protocols and 30 PCR cycles were done with extension temperature of 72°C.

Transformation of *A. thaliana* with transformed *A. tumefaciens*

The optical density of the *A. tumefaciens* bacterial culture was adjusted to 0.7 with infiltration medium (5% sucrose, 0.05% Silwett L-77 surfactant). *A. thaliana* cv. Columbia plants (6 punnets per construct and 10-12 plants per punnet) were pruned by removing secondary bolts. Pruned *A. thaliana* plants in punnets were dipped into infiltration solution and moved back and forth for 5 seconds. Punnets were put on their side to allow excess infiltration medium to drain covered with a top tray and wrapped in plastic wrap to maintain humidity. Plants were placed in a growth room at ambient conditions for 24 hours. After this period, the top tray and plastic wrap were removed and plants were set upright until siliques formed.

Seeds were harvested and sterilized with a 5% sodium hypochlorite solution to destroy any residual *A. tumefaciens* bacteria and fungal contamination.

Under sterile conditions, 100 µl seeds from the transformed *A. thaliana* plants were placed into an Eppendorf tube. One ml sterile water was added and the seeds left to imbibe the water for no longer than an hour. The water was remove by centrifugation, 1 ml 70% ethanol added to the seeds and gently mixed. This step was not allowed to last longer than one minute. The ethanol was removed by centrifugation, 1 ml 5% sodium hypochlorite solution was added to the seeds and gently mixed for up to 5 min. The sodium hypochlorite solution was removed by centrifugation and the seeds washed with sterile water for 1 min. The washing step was repeated three more times with centrifugation. Seeds were finally resuspended in sterile water. 500 µl of seeds in solution were pipetted onto half-strength Murashige and Skoog medium (MS; Gibco BRL) agar plates containing 50 mg/l kanamycin and 250 mg/l timentin and spread evenly with a flamed wire-loop. The Petri dishes were placed in a refrigerator for 3 days to allow the seeds to stratify. Thereafter the plates were placed in growth room and grown under lights at 22°C with a 14 hour photoperiod until

germination. Putative transformant seedlings were selected as those growing on the antibiotic-containing medium, with large, healthy-looking dark green leaves and a strong root system. These transgenic plants were removed and placed into soil culture at 22°C with a 12 hour photoperiod.

Staining of plant tissues

Tissue were taken from the flower, leaf, stem and root of *A. thaliana* transformed with constructs of *P. radiata* unknown pollen specific promoter and *E. grandis* pollen specific promoter and stained histochemically to determine the expression of the GUS gene under control of the pollen specific promoters. The GUS staining protocol is described by Campisi *et al.*, *Plant J.* 17:699-707, 1999.

A. thaliana flower, leaf, stem and root tissue were immersed in staining solution (50 mM $NaPO_4$ pH 7.2; 0.5% Triton X-100; 1 mM X Glucuronide sodium salt (Gibco BRL)) for immunochemical staining. Vacuum was applied twice for 5 min to infiltrate the tissue with the staining solution. The tissue was left in the staining solution for 2 days (with agitation) at 37° for color development and then destained in 70% ethanol for 24 hours at 37°C (with agitation). The tissues were examined for blue GUS staining using a light microscope. GUS expression was observed only in the flower buds of plants transformed with the *P. radiata* pollen specific promoter construct, and not in the leaf, stem or root tissue. With the *E. grandis* pollen specific promoter construct, Gus expression was observed in the floral buds as well as in the hydathodes of the leaves. No expression was observed in the stem or root tissues.

To determine in which cell layers the GUS gene was expressed, flower buds were fixed for thin sectioning. The flower buds were fixed with formaldehyde acetic acid (FAA) in an Eppendorf tube and vacuum was applied twice for 15 min. After incubation for 2 hours at room temperature, vacuum was again applied for 15 min and the tissue left overnight at 4°C. The tissues were then dehydrated using a series of ethanol and then passed into a xylene series. Paraffin wax (Sigma) was added slowly and the tissues left for 72 hours with wax changes every 12 hours. Sections of 8 to 10 µm thickness were prepared using a microtome.

The thin sections illustrated that GUS expression was restricted to the tapetum cell layer in the anther of the floral bud of *A. thaliana* transformed with the *P. radiata* construct (SEQ ID NO: 49). No staining was observed in other tissues from the floral bud. GUS expression was confined to the pollen grains within the flower bud of *A. thaliana*

transformed with the *E. grandis* pollen specific promoter construct, with low levels of GUS expression in the fibrous and connective tissue of the anther. No GUS expression was observed in other organs of the floral bud.

EXAMPLE 19

Determination of the Activity of an *E. grandis* EF1 alpha Promoter Deletion Construct in transformed *Arabidopsis thaliana* cv Columbia

Protoplasts from *Nicotiana tabacum* Bright Yellow 2 (BY-2) cell suspension were transformed with a deletion construct of the *E. grandis* EF1-alpha promoter to determine GUS expression. Base pairs 2,174 to 3,720 of SEQ ID NO: 127 were cloned into expression vector pART9, containing the reporter gene GUS and an OCS termination sequence.

Preparation of protoplasts

Sterile *Nicotiana tabacum* Bright Yellow-2 (BY-2) suspension cultures were prepared as described in Example 17. After incubation for 3 to 5 days, 3 g of the *N. tabacum* BY-2 cell suspension were suspended in an enzyme solution containing 1% cellulase, 0.3% pectinase and 0.5% driselase in 0.4 M mannitol. These were left to digest in the dark, with agitation at 26° C, for 3-4 hours. Protoplasts were purified by filtration through a 63 µm nylon mesh. Protoplasts were centrifuged at 80x g for 5 min, washed twice with 10 ml FMS medium (Fukuda, Murashige and Skoog medium; Hasezawa & Syono, *Plant Cell Physiol.* 24:127-132, 1983) and finally resuspended in 5 ml FMS medium. Protoplasts were counted in a hemocytometer and viability determined by staining with 5 mg/ml FDA (fluorescein deacetate; Sigma St Louis MI) in 100% acetone by viewing under the fluorescent microscope.

Transformation of Protoplasts

Protoplasts were transformed according to the protocol described by Morgan and Ow (In: Methods in Plant Molecular Biology: a laboratory course manual, pp. 1-16. P. Maliga, D.Klessig, A.R. Cashmore, W. Gruissem, and J.E.Varner, eds. Cold Spring Harbor Laboratory, CSHP, NY). Briefly, the protocol is as follows. Following the counting step, protoplasts were centrifuged at 80x g for 5 min and resuspended in 1x MaMg solution (0.4 M manniotol, 15 mM $MgCl_2.6H_2O$, 0.1% 2-(*N*-Morpholino)ethane sulfonic acid (MES)) to a density of $5x10^6$ protoplasts/ml. Aliquots of 100 µl (0.5 x 10^5 protoplasts) were distributed to

15 ml tubes and washed with 5 ml 1x MaMg (200g, 5 min). Pelleted protoplasts were resuspended in 500ul 1x MaMg solution, and heat shocked by placing at 45°C for 5 minutes. After incubation at room temperature 5-10 minutes, the transforming DNA was added (10-20 μg DNA + 10 μg carrier DNA). To this, 500 μl 40% PEG-3500 was gently added and incubated for 25 minutes at room temperature. 5ml W5 (154 mM NaCl, 125 mM $CaCl_2.2H_2O$, 5 mM KCl, 5 mM Glucose) solution was slowly added stepwise followed by centrifugation at 200x g for 5 min. Pelleted protoplasts were resuspended in 1ml K3AM medium at approximately 0.5 x 10^5 protoplasts/ml. Samples were transferred to 6-well plates and incubated in the dark at 26°C for 48 hours.

To extract protein, protoplasts were centrifuged at 200x g for 5 min in a microfuge, resuspended in 100 μl GUS extraction buffer (50 mM $NaPO_4$ pH 7.2, 10 mM EDTA pH 8, 0.01% Sarcosyl, 0.1% Triton X-100) containing β-mercaptoethanol (Jefferson et al., *Plant Mol. Biol. Rep.* 5:387-405, 1987) and vortexed for 1 min. The homogenate was cleared by centrifugation at 5,000 rpm for 5 minutes. The supernatant containing the protein was transferred to a fresh tube and stored at -80°C. The protein concentrations were determined by BioRad protein assay kit (BioRad, Hercules, CA) following the manufacturer's protocols. Protein extracts were diluted 1/10 with extraction buffer.

Determination of GUS expression

GUS expression in the protoplast extracts was determined using a MUG (4-methyl umbelliferyl β-D-glucuronide) assay. Protein samples, containing 1 μg protein made up to a total volume of 45 μl with extraction buffer, were aliquoted onto a microtitre plate and incubated at 37°C. To each sample, 5 μl of 10 mM MUG was added so that the final concentration of MUG was 1 mM. The plate was incubated at 37°C for 30 min and terminated by adding 150 μl stop solution (0.2 M Na_2CO_3, pH 11.20), still keeping the plates at 37°C. Plates were read in a Victor^2 1420 Multilabel counter with excitation set at 365 nm and emission at 455 nm. The concentration of 4-methyl-umbelliferone (MU) was calculated against a standard curve and the GUS expression calculated.

In Fig. 4, increased expression of the GUS reporter gene in *N. tabacum* BY-2 protoplasts transformed with an *E. grandis* EF1 alpha deletion construct was seen compared to the control plasmid without an insert.

EXAMPLE 20

Determination of the Effect of the 3'UTR Super-ubiquitin (SU) Sequences on Gene Expression in *Arabidopsis thaliana* cv Columbia

In the polynucleotide sequences given in SEQ. ID NO: 1 encoding *P. radiata* superubiquitin (SU) promoter and gene sequences, an 3' untranslated region (UTR) was identified (nucleotides 1,754 to 3,083). To determine the effect of this region on the expression of genes, 250 bp of the 3' UTR (nucleotides 2,755 to 3,073 from SEQ ID NO: 1) was cloned in the sense and antisense orientation into plasmid pBI-121 containing the GUS gene under control of the 35S CaMV promoter and plasmid pBI-101 containing the GUS gene under control of the *P. radiata* SU promoter (including the intron) given in SEQ ID NO: 2. For controls, constructs were made that contained the SU promoter without an intron (SEQ ID NO: 3) and without the SU 3' UTR sequence, the SU promoter with an intron (SEQ ID NO: 2) and without the SU 3' UTR sequence as well as a construct containing the 35S CaMV promoter but not the SU 3' UTR sequence.

A. thaliana cv Columbia were transformed with these constructs using the floral dip protocol described in Example 18.

Determining the level of gene expression using a MUG assay.

Six *A. thaliana* plants were harvested by trimming off the dried tissue and then harvesting the rest of the plant, including the roots. The roots were rinsed in tap water and the samples immersed in liquid nitrogen before storing at -80°C. Six plants from each construct were ground under liquid nitrogen and approximately 100 mg transferred to a microfuge tube. Five samples from each control were included in the assay. Extraction buffer (50 mM $NaPO_4$ pH 7.2, 10 mM EDTA pH 8, 0.01% Sarcosyl, 0.1% Triton X-100) was prepared. To 32 ml of extraction buffer, 8 ml methanol and 28 μl β-mercaptoethanol was added. Of this buffer, 200 μl was added to each sample, vortexed and stored on ice. Samples were spun at 4°C at 15, 000 rpm for 15 min. The supernatant was transferred to a fresh tube and diluted with 800 μl of extraction buffer. Protein concentration was determined using the BioRad Protein Assay Kit.

The expression of GUS by the four constructs was determined using a MUG assay, as follows. To 28 ml extraction buffer (as described in Example 18), 8 ml methanol, 56 μl β-mercaptoethanol and 4 ml of 10 mg/ml bovine serum albumin (BSA) were added. To

microtitre plate wells, 100 and 10 ng of protein from each construct was added as well as 25 μl extraction buffer containing BSA and 5 μl 10 mM MUG. The plate was covered in foil and incubated at 37°C for exactly 20 minutes. The reaction was terminated by adding 150 μl 0.2 M Na_2CO_3 pH 11.2. Plates were read with a Victor^2 1420 Multilabel counter with excitation set at 365 nm and emission at 455 nm. GUS expression levels were determined against a MU standard curve.

In Fig. 5, construct SR34 containing the SU 3'UTR in the sense orientation enhanced the expression of the SU without intron promoter almost to the level of the SU promoter with the intron. In constructs SR33 and SR35 containing the 3'UTR in the antisense orientation, promoter activity was reduced to basal levels.

Claims:

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:
   (a) sequences recited in SEQ ID NO: 2-14, 20, 22-33, 35-43, 45-49, 51, 52, 56-62 and 88-127;
   (b) complements of the sequence recited in SEQ ID NO: 2-14, 20, 22-33, 35-43, 45-49, 51, 52, 56-62 and 88-127;
   (c) reverse complements of the sequence recited in SEQ ID NO: 2-14, 20, 22-33, 35-43, 45-49, 51, 52, 56-62 and 88-127;
   (d) reverse sequences of the sequences recited in SEQ ID NO: 2-14, 20, 22-33, 35-43, 45-49, 51, 52, 56-62 and 88-127;
   (e) sequences having at least 40% identical nucleotides to a sequence provided in SEQ ID NO: 2-14, 20, 22-23, 25-33, 35-42, 45-49, 57-59, 62, 88-99, 101-112 and 114-127 as determined using the computer algorithm BLASTN;
   (f) sequences having at least 60% identical nucleotides to a sequence provided in SEQ ID NO: 2-14, 20, 22-23, 25-33, 35-42, 45-49, 56-59, 62, 88-99, 101-112 and 114-127 as determined using the computer algorithm BLASTN;
   (g) sequences having at least 75% identical nucleotides to a sequence provided in SEQ ID NO: 2-14, 20, 22-23, 25-33, 35-49, 52, 56-61, 62, 88-99, 101-112 and 114-127 as determined using the computer algorithm BLASTN; and
   (h) sequences having at least 90% identical nucleotides to a sequence provided in SEQ ID NO: 2-14, 20, 22-33, 35-49, 51, 52, 56-61, 62, 88-112 and 114-127 as determined using the computer algorithm BLASTN.
2. An isolated polynucleotide comprising a sequence selected from the group consisting of:
   (a) sequences recited in SEQ ID NO: 1 and 34;
   (b) complements of sequences recited in SEQ ID NO: 1 and 34;
   (c) reverse complements of sequences recited in SEQ ID NO: 1 and 34;
   (d) reverse sequences of sequences recited in SEQ ID NO: 1 and 34;
   (e) sequences having at least 40% identical nucleotides to sequences recited in SEQ ID NO: 1 and 34 as determined using the computer algorithm BLASTN;
   (f) sequences having at least 60% identical nucleotides to sequences recited in SEQ ID NO: 1 and 34 as determined using the computer algorithm BLASTN;

(g) sequences having at least 75% identical nucleotides to sequences recited in SEQ ID NO: 1 and 34 as determined using the computer algorithm BLASTN; and

(h) sequences having at least 90% identical nucleotides to sequences recited in SEQ ID NO: 1 and 34 as determined using the computer algorithm BLASTN.

3. An isolated polypeptide encoded by a polynucleotide selected from the group consisting of:

(a) sequences recited in SEQ ID NO: 1, 22, 25, 26, 28, 34, 35, 36, 40, 45, 46, 51-53, 56, 57, 60, 61, 86 and 124;

(b) complements of the sequences of SEQ ID NO: 1, 22, 25, 26, 28, 34, 35, 36, 40, 45, 46, 51-53, 56, 57, 60, 61, 86 and 124;

(c) reverse complements of a sequence of SEQ ID NO: 1, 22, 25, 26, 28, 34, 35, 36, 40, 45, 46, 51-53, 56, 57, 60, 61, 86 and 124;

(d) reverse sequences of a sequence of SEQ ID NO: 1, 22, 25, 26, 28, 34, 35, 36, 40, 45, 46, 51-53, 56, 57, 60, 61, 86 and 124;

(e) sequences having at least 40% identical nucleotides to a sequence provided in SEQ ID NO: 1, 22, 25, 26, 28, 34, 35, 36, 40, 45, 46, 51-53, 56, 57, 60, 61, 86 and 124;

(f) sequences having at least 60% identical nucleotides to a sequence provided in SEQ ID NO: 1, 22, 25, 26, 28, 34, 35, 36, 40, 45, 46, 51-53, 56, 57, 60, 61, 86 and 124;

(g) sequences having at least 75% identical nucleotides to a sequence provided in SEQ ID NO: 1, 22, 25, 26, 28, 34, 35, 36, 40, 45, 46, 51-53, 56, 57, 60, 61, 86 and 124; and

(h) sequences having at least 90% identical nucleotides to a sequence provided in SEQ ID NO: 1, 22, 25, 26, 28, 34, 35, 36, 40, 45, 46, 51-53, 56, 57, 60, 61, 86 and 124.

4. The isolated polypeptide of claim 3, wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 63-80, 87 and 130.

5. A genetic construct comprising a polynucleotide according to any one of claims 1 and 2.

6. A genetic construct comprising, in the 5'-3' direction:

(a) a promoter sequence,

(b) a DNA sequence of interest; and

(c) a gene termination sequence,

wherein the promoter sequence comprises an isolated polynucleotide according to claim 1.

7. The genetic construct of claim 6, wherein the DNA sequence of interest comprises an open reading frame encoding a polypeptide of interest.

8. The genetic construct of claim 6, wherein the DNA sequence of interest comprises a non-coding region of a gene encoding a polypeptide of interest.

9. A transgenic cell comprising a genetic construct of any one of claims 5-8.

10. An organism comprising a transgenic cell according to claim 9.

11. A plant comprising a transgenic cell according to claim 9, or a part or propagule or progeny thereof.

12. A method for modifying gene expression in a target organism comprising stably incorporating into the genome of the organism a genetic construct according to any one of claims 5-8.

13. The method of claim 12 wherein the organism is a plant.

14. A method for producing a plant having modified gene expression comprising:

(a) transforming a plant cell with a genetic construct to provide a transgenic cell, wherein the genetic construct comprises: (i) a promoter sequence comprising a sequence of SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127; (ii) a DNA sequence of interest; and (c) a gene termination sequence; and

(b) cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth.

15. A method for modifying a phenotype of a target organism, comprising stably incorporating into the genome of the target organism a genetic construct comprising:

(a) a promoter sequence comprising a sequence of SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127;

(b) a DNA sequence of interest; and

(c) a gene termination sequence.

16. The method of claim 15, wherein the target organism is a plant.

17. A method for identifying a gene responsible for a desired function or phenotype, comprising:

(a) transforming a plant cell with a genetic construct comprising a promoter sequence operably linked to a gene to be tested, the promoter sequence comprising a sequence of SEQ ID NO: 1-14, 20, 22-62, 81-86 and 88-127;

(b) cultivating the plant cell under conditions conducive to regeneration and mature plant growth to provide a transgenic plant; and

(c) comparing the phenotype of the transgenic plant with the phenotype of non-transformed plants.

18. An isolated polynucleotide comprising a sequence selected from the group consisting of:

(a) a sequence recited in SEQ ID NO: 21;

(b) complements of a sequence recited in SEQ ID NO: 21;

(c) reverse complements of a sequence recited in SEQ ID NO: 21;

(d) reverse sequences of a sequence recited in SEQ ID NO: 21;

(e) sequences having at least 40% identical nucleotides to a sequence recited in SEQ ID NO: 21 as determined using the computer algorithm BLASTN;

(f) sequences having at least 60% identical nucleotides to a sequence recited in SEQ ID NO: 21 as determined using the computer algorithm BLASTN;

(g) sequences having at least 75% identical nucleotides to a sequence recited in SEQ ID NO: 21 as determined using the computer algorithm BLASTN; and

(h) sequences having at least 90% identical nucleotides to a sequence recited in SEQ ID NO: 21 as determined using the computer algorithm BLASTN.

19. A genetic construct comprising a polynucleotide according to claim 18.

20. A transgenic cell comprising a genetic construct according to claim 19.

21. A method for modifying gene expression in a target organism comprising stably incorporating into the genome of the organism a genetic construct according to claim 19.

22. A method for modifying expression of a polynucleotide that comprises the sequence of SEQ ID NO: 21, the method comprising removing the sequence of SEQ ID NO: 21 from the polynucleotide.

23. A polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 2-14, 20, 22-33, 35-43, 45-49, 51, 52, 56-62 and 88-127 operably linked to a heterologous polynucleotide.

24. The polynucleotide of claim 23, wherein the heterologous polynucleotide comprises an open reading frame.

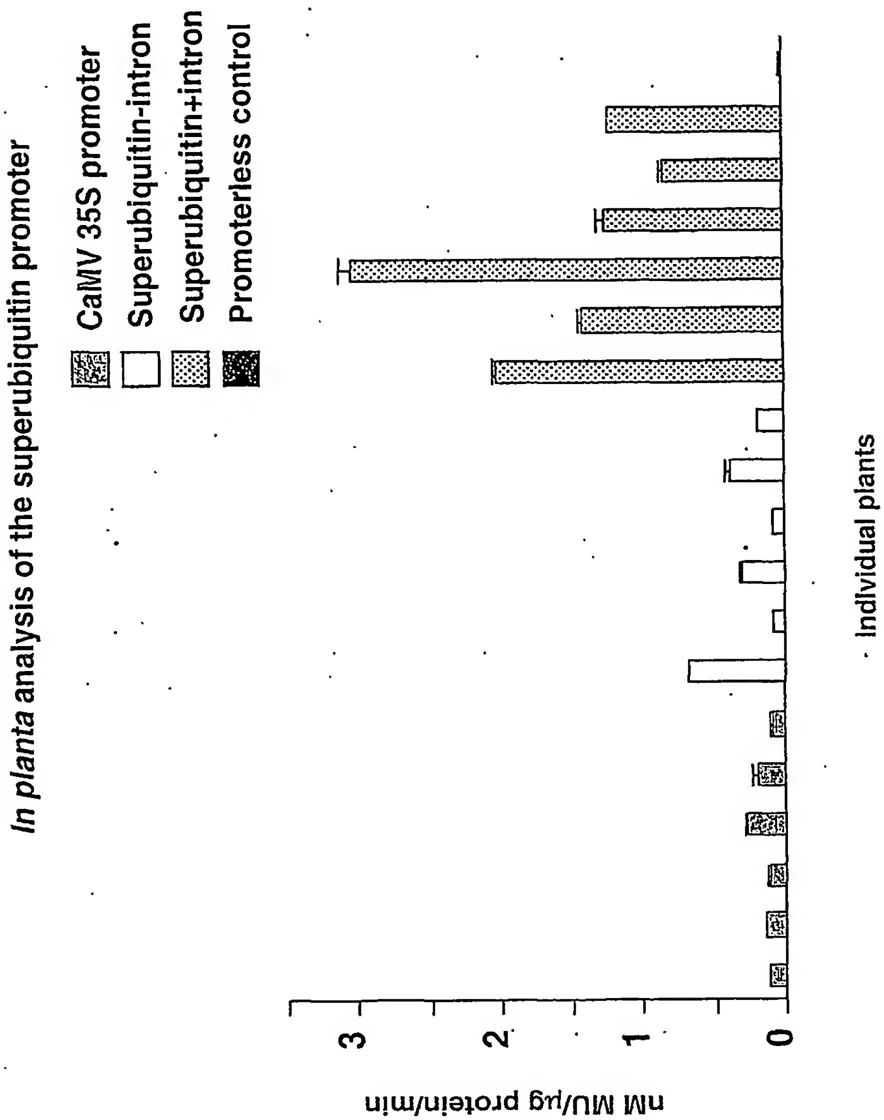
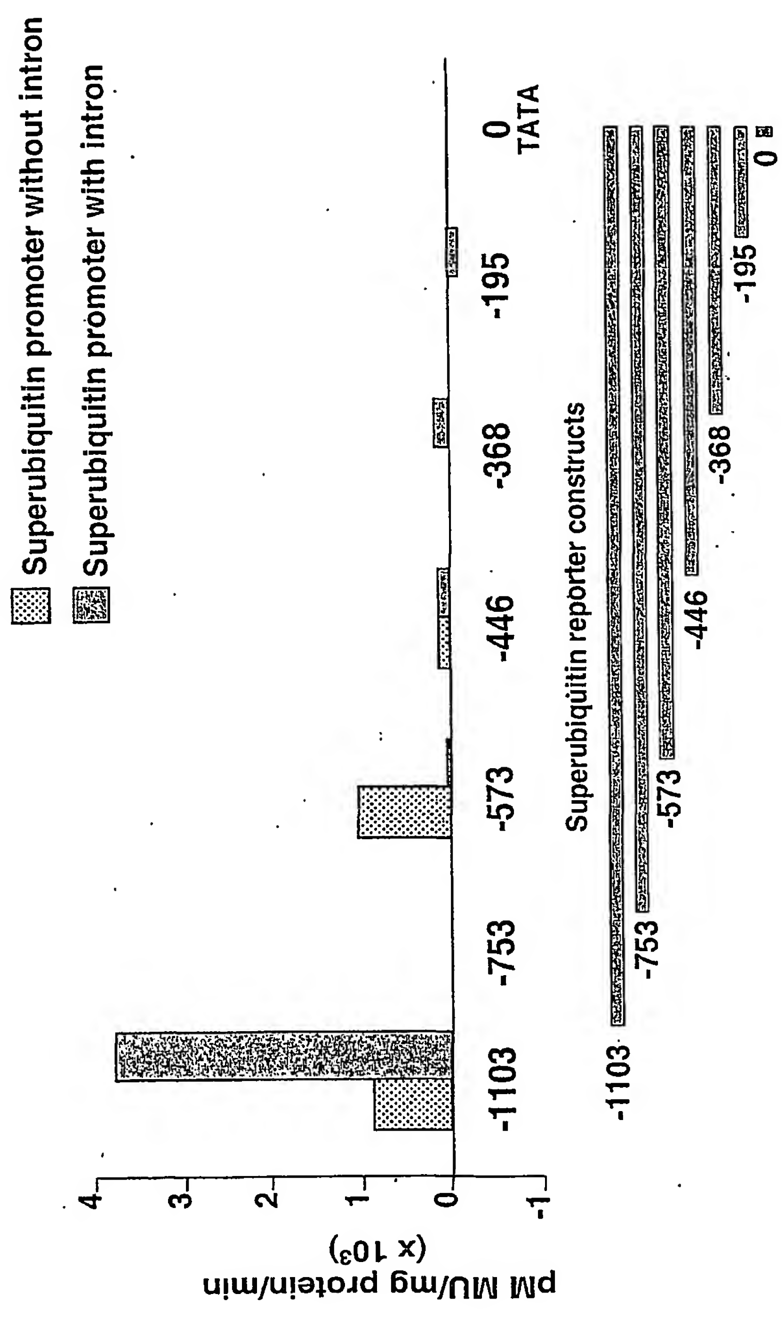

In planta analysis of the superubiquitin promoter
CaMV 35S promoter
Superubiquitin-intron
Superubiquitin+intron
Promoterless control
nM MU/µg protein/min
0
1
2
3
Individual plants

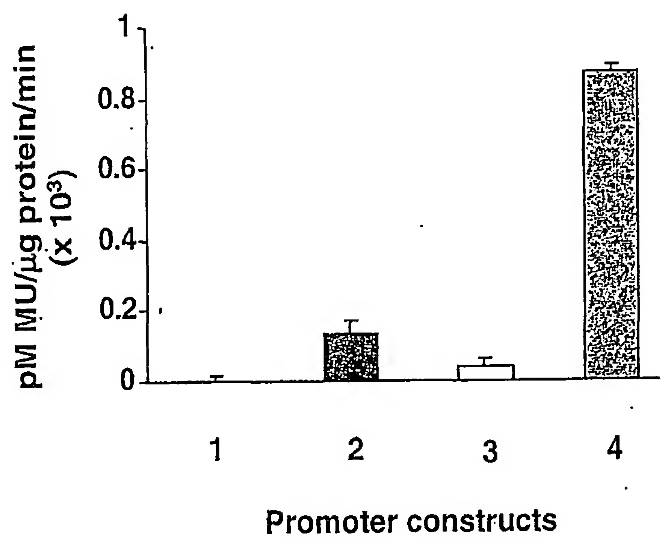
Figure 1

Figure 2

In vitro analysis of the superubiquitin promoter using deletion constructs



In vitro analysis of *P. radiata* constitutive promoters



1 pBI-101

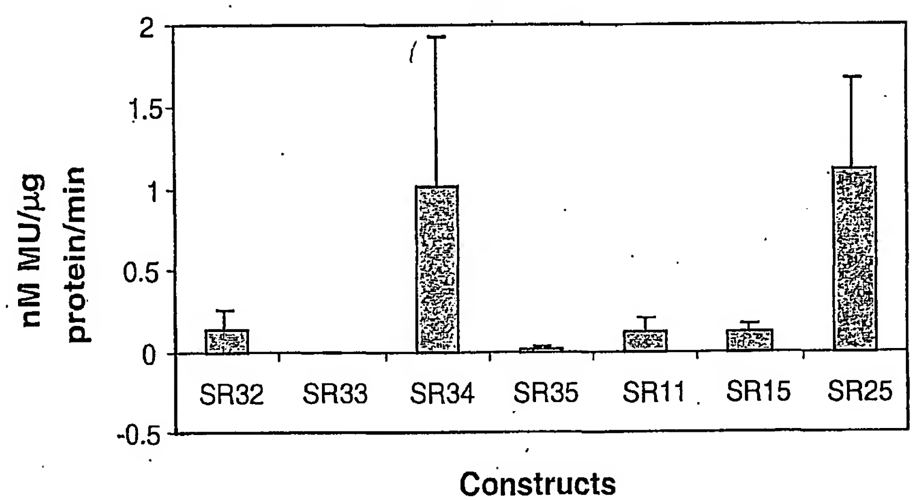
2 Elongation Factor 1α

3 5-Adenosylmethionine synthetase

4 Superubiquitin promoter without intron

Figure 3

Determination of GUS expression levels in *A. thaliana* cv Columbia by constructs containing Super-ubiquitin (SU) 3' UTR sequence in sense and antisense orientation



SR32 35S CaMV promoter with sense 3' UTR

SR33 35S CaMV promoter with antisense 3' UTR

SR34 *P. radiata* SU promoter with sense 3' UTR

SR35 *P. radiata* SU promoter with antisense 3'UTR

SR11 Superubiquitin promoter without intron

SR15 35S CaMV promoter

SR25 Superubiquitin promoter with intron

Figure 4

Expression of GUS reporter gene under control of *E. grandis* EF1 alpha promoter deletion construct in *N. tabacum* BY-2 protoplasts

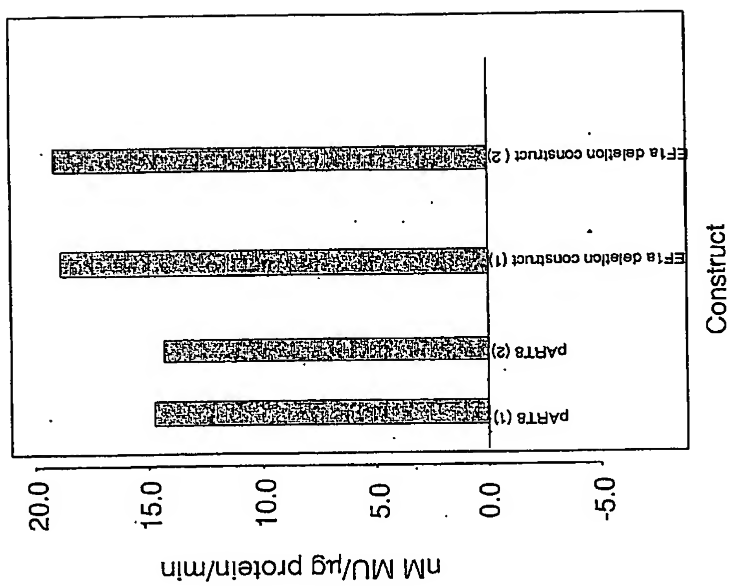


Figure 5

SEQUENCE LISTING

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Rice, Stephen
Eagleton, Clare
Lasham, Annette

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                           Met Gln Ile Phe Val Lys Thr Leu Thr
                           1               5

ggt aag acc atc act ctc gag gtc gag agc tct gac acc att gac aat      2139
Gly Lys Thr Ile Thr Leu Glu Val Glu Ser Ser Asp Thr Ile Asp Asn
10                  15                  20                  25

gtt aaa gct aag atc cag gac aag gaa ggg att ccc ccc gac cag cag      2187
Val Lys Ala Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln
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Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ala
            45                  50                  55

gat tac aac atc cag aaa gaa tct acc ctc cac ctt gtt ctc cgt ttg      2283
Asp Tyr Asn Ile Gln Lys Glu Ser Thr Leu His Leu Val Leu Arg Leu
        60                  65                  70

agg ggt ggc atg caa atc ttt gta aaa aca cta act gga aag aca att      2331
Arg Gly Gly Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile
    75                  80                  85

aca ttg gaa gtt gag agc tcg gac acc att gac aac gtc aag gcc aag      2379
Thr Leu Glu Val Glu Ser Ser Asp Thr Ile Asp Asn Val Lys Ala Lys
90                  95                  100                 105

atc cag gac aag gaa gga att ccc cct gac cag cag agg ctt atc ttc      2427
Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe
                110                 115                 120

gct ggt aag cag ctg gag gat ggc agg acc ttg gct gat tac aat att      2475
Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu Ala Asp Tyr Asn Ile
            125                 130                 135

caa aag gaa tcg acc ctg cat ttg gtg ctt cgt cta aga gga ggc atg      2523
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      140                 145                 150

caa atc ttt gtg aaa acc ctt aca ggt aaa acc att act ctg gaa gtg      2571
Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu Val
   155                 160                 165

gaa agc tcg gac acc att gac aat gtg aag gct aag atc cag gac aag      2619
Glu Ser Ser Asp Thr Ile Asp Asn Val Lys Ala Lys Ile Gln Asp Lys
170                 175                 180                 185

gag gga att cca cct gac cag cag agg ttg atc ttt gcc ggt aag cag      2667
Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln
                190                 195                 200

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            205                 210                 215

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Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Phe
      220                 225

tgttatttgt ggataataaa ttcgggtgat gttcagtgtt tgtcgtattt ctcacgaata    2821
aattgtgttt atgtatgtgt tagtgttgtt tgtctgtttc agaccctctt atgttatatt    2881
tttcttttcg tcggtcagtt gaagccaata ctggtgtcct ggccggcact gcaataccat    2941
ttcgtttaat ataaagactc tgttatccgt tatgtaattc catgttatgt ggtgaaatgt    3001
ggatgaaatt cttagaaatt attattgtaa tttgaaactt ccttcgtcaa taatctgcac    3061
aacacattta ccaaaaaaaa aa                                             3083
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<210> 2
<211> 2064
<212> DNA
<213> Pinus radiata

<220>
<221> 5'UTR
<222> (1)...(2064)

<221> intron
<222> (1196)...(2033)

<400> 2

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aaaacccctc acaaatacat aaaaaaaatt ctttatttaa ttatcaaact ctccactacc      60
tttcccacca accgttacaa tcctgaatgt tggaaaaaac taactacatt gatataaaaa     120
aactacatta cttcctaaat catatcaaaa ttgtataaat atatccactc aaaggagtct     180
agaagatcca cttggacaaa ttgcccatag ttggaaagat gttcaccaag tcaacaagat     240
ttatcaatgg aaaaatccat ctaccaaact tactttcaag aaaatccaag gattatagag     300
taaaaaatct atgtattatt aagtcaaaaa gaaaaccaaa gtgaacaaat attgatgtac     360
aagtttgaga ggataagaca ttggaatcgt ctaaccagga ggcggaggaa ttccctagac     420
agttaaaagt ggccggaatc ccggtaaaaa agattaaaat ttttttgtag agggagtgct     480
tgaatcatgt tttttatgat ggaaatagat tcagcaccat caaaaacatt caggacacct     540
aaaatttga agtttaacaa aaataacttg gatctacaaa aatccgtatc ggattttctc      600
taaatataac tagaattttc ataactttca aagcaactcc tcccctaacc gtaaaacttt     660
tcctacttca ccgttaatta cattccttaa gagtagataa agaaataaag taaataaaag     720
tattcacaaa ccaacaattt atttctttta tttacttaaa aaaacaaaaa gtttatttat     780
tttacttaaa tggcataatg acatatcgga gatccctcga acgagaatct tttatctccc     840
```

```
tggttttgta ttaaaaagta atttattgtg gggtccacgc ggagttggaa tcctacagac      900
gcgctttaca tacgtctcga gaagcgtgac ggatgtgcga ccggatgacc ctgtataacc      960
caccgacaca gccagcgcac agtatacacg tgtcatttct ctattggaaa atgtcgttgt     1020
tatccccgct ggtacgcaac caccgatggt gacaggtcgt ctgttgtcgt gtcgcgtagc     1080
gggagaaggg tctcatccaa cgctattaaa tactcgcctt caccgcgtta cttctcatct     1140
tttctcttgc gttgtataat cagtgcgata ttctcagaga gcttttcatt caaaggtatg     1200
gagttttgaa gggctttact cttaacattt gtttttcttt gtaaattgtt aatggtggtt     1260
tctgtggggg aagaatcttt tgccaggtcc ttttgggttt cgcatgttta tttgggttat     1320
ttttctcgac tatggctgac attactaggg ctttcgtgct ttcatctgtg ttttcttccc     1380
ttaataggtc tgtctctctg gaatatttaa ttttcgtatg taagttatga gtagtcgctg     1440
tttgtaatag gctcttgtct gtaaaggttt cagcaggtgt ttgcgtttta ttgcgtcatg     1500
tgtttcagaa ggcctttgca gattattgcg ttgtacttta atattttgtc tccaaccttg     1560
ttatagtttc cctcctttga tctcacagga accctttctt ctttgagcat tttcttgtgg     1620
cgttctgtag taatatttta attttgggcc cgggttctga gggtaggtga ttattccagt     1680
gatgtgcttt ccctataagg tcctctatgt gtaagctgtt agggtttgtg cgttactatt     1740
gacatgtcac atgtcacata ttttcttcct cttatccttc gaactgatgg ttctttttct     1800
aattcgtgga ttgctggtgc catattttat ttctattgca actgtatttt agggtgtctc     1860
tttctttttg atttcttgtt aatatttgtg ttcaggttgt aactatgggt tgctagggtg     1920
tctgccctct tcttttgtgc ttctttcgca gaatctgtcc gttggtctgt atttgggtga     1980
tgaattattt attccttgaa gtatctgtct aattagcttg tgatgatgtg caggtatatt     2040
cgttagtcat atttcaattt caag                                            2064
```

<210> 3
<211> 1226
<212> DNA
<213> Pinus radiata

<220>
<221> 5'UTR
<222> (1)...(1266)

<400> 3

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aaaacccctc acaaatacat aaaaaaaatt ctttatttaa ttatcaaact ctccactacc       60
tttcccacca accgttacaa tcctgaatgt tggaaaaaac taactacatt gatataaaaa      120
aactacatta cttcctaaat catatcaaaa ttgtataaat atatccactc aaaggagtct      180
agaagatcca cttggacaaa ttgcccatag ttggaaagat gttcaccaag tcaacaagat      240
ttatcaatgg aaaaatccat ctaccaaact tactttcaag aaaatccaag gattatagag      300
taaaaaatct atgtattatt aagtcaaaaa gaaaaccaaa gtgaacaaat attgatgtac      360
aagtttgaga ggataagaca ttggaatcgt ctaaccagga ggcggaggaa ttccctagac      420
agttaaaagt ggccggaatc ccggtaaaaa agattaaaat ttttttgtag agggagtgct      480
tgaatcatgt tttttatgat ggaaatagat tcagcaccat caaaaacatt caggacacct      540
aaaattttga agtttaacaa aaataacttg gatctacaaa aatccgtatc ggattttctc      600
taaatataac tagaattttc ataactttca aagcaactcc tcccctaacc gtaaaacttt      660
tcctacttca ccgttaatta cattccttaa gagtagataa agaaataaag taaataaaag      720
tattcacaaa ccaacaattt atttctttta tttacttaaa aaaacaaaaa gtttatttat      780
tttacttaaa tggcataatg acatatcgga gatccctcga acgagaatct tttatctccc      840
tggttttgta ttaaaaagta atttattgtg gggtccacgc ggagttggaa tcctacagac      900
gcgctttaca tacgtctcga gaagcgtgac ggatgtgcga ccggatgacc ctgtataacc      960
caccgacaca gccagcgcac agtatacacg tgtcatttct ctattggaaa atgtcgttgt     1020
tatccccgct ggtacgcaac caccgatggt gacaggtcgt ctgttgtcgt gtcgcgtagc     1080
gggagaaggg tctcatccaa cgctattaaa tactcgcctt caccgcgtta cttctcatct     1140
tttctcttgc gttgtataat cagtgcgata ttctcagaga gcttttcatt caaaggtata     1200
ttcgttagtc atatttcaat ttcaag                                          1226
```

<210> 4
<211> 485
<212> DNA

<213> Pinus radiata

<220>
<221> 5'UTR
<222> (1)...(431)

<221> TATA_signal
<222> (350)...(356)

<221> CAAT_signal
<222> (326)...(333)

<400> 4
agtaaaattg gcccatgtag gactaagtca aaatcaaaat tccatctcta aaagcggaac 60
tttgtcccct gaaaattttg actaatttcc aaccaaaaaa aagtggggga aaatataaaa 120
ctctaactaa taaaacaata atcaccaaaa atctatcacc aaaaatgaaa aaagattttg 180
aatactaggc catatgagct acacaaattt caaaagtatc ttacacttat tacgcacccg 240
gatgtcccca ctttcgaaaa acccgtttca agcctttcac gaaagtccaa cggtcagaaa 300
attcaaaatg actgtttgag gcagagccaa tctaggacca cgctccattt atatatggcc 360
tctgcttctc tcgaccctta gagtcctctg ctctgcgaat cttgttgtta gttactgtgt 420
acgctgtaac aatggatgcc tatgagaagt tggagaaggt gggagaagga acctatggga 480
aggtg 485

<210> 5
<211> 246
<212> DNA
<213> Pinus radiata

<220>
<221> 5'UTR
<222> (1)...(167)

<221> TATA_signal
<222> (185)...(191)

<400> 5
tgagaacatg ataagctgtg taaattcatg ctagtcacca taacttttct cattgctttt 60
catccacact gttgattcat tcattatata agatcagatt cgtatgatat acaggcaacc 120
atagaaacaa ccagcaaagt tactagcagg aaatccaact aggtatcatg aagactacca 180
acgcaggctc gataatgttg gtgctcatta tttttgggtg ctgtttcatt ggggtcatag 240
ctacat 246

<210> 6
<211> 600
<212> DNA
<213> Pinus radiata

<220>
<221> 5'UTR
<222> (1)...(167)

<221> TATA_signal
<222> (471)...(477)

<221> CAAT_signal
<222> (444)...(451)

<400> 6
caccaattta atgggatttc agatttgtat cccatgctat tggctaagcc atttttctta 60
ttgtaatcta accaattcca atttccaccc tggtgtgaac tgactgacaa atgcggcccg 120
aaaacagcga atgaaatgtc tgggtgatcg gtcaaacaag cggtgggcga gagaacgcgg 180
gtgttggcct agccgggatg gggtaggta gacggcgtat taccggcgag ttgtccgaat 240
ggagttttcg gggtaggtag taacgtagac gtcaatggaa aaagtcataa tctccgtcaa 300
aaatccaacc gctccttcac accgcagagt tggtggccac gggaccctcc acccactcac 360
tcaatcgatc gcctgccgtg gttgcccatt attcaaccat acgccacttg actcttcacc 420
aacaattcca ggccggcttt cgagacaatg tactgcacag gaaaatccaa tataaaaggc 480
cggcctccgc ttccttctca gtagccccca gctcattcaa ttcttcccac tgcaggctac 540
atttgtcaga cacgttttcc gccatttttc gcctgtttct gcggagaatt tgatcaggtt 600

<210> 7
<211> 591
<212> DNA
<213> Eucalyptus grandis

<220>
<221> 5'UTR
<222> (1)...(591)

<221> TATA_signal
<222> (432)...(437)

<400> 7
agtttggaat gtgttgtgtg tgatgtgatg gagagtatca gcattccaaa catgacatgg 60
ttttaactta tctgcaatgg tttctttttt attcagcgaa ctcgatggct gatgctgaga 120
gaaatgaatt gggaagtcga tcgacaatgg cagctcaact caatgatcct caggtataag 180
catttttttg gcagctctgg tcattgtgtc ttcaactttt agatgagagc aaatcaaatt 240
gactctaata ccggttatgt gatgagtgaa tcatttgctt ttagtagctt taatttatgc 300
ccccatctta gttgggtata aaggttcaga gtgcgaagat tacatctatt ttggttcttg 360
caggacacag ggattcatgc tagacacatc agcagtgttt ctacgttgga tagtggtatg 420
tacttagcta ctataaagga aattttgata gatatgtttg atatggtgct tgtacagatc 480
tatttaatgt caatgtattt gaaactatct tgtctcataa ctttcttgaa gaatacaatg 540
atgagactgg gaaccctatc tggaagaata gagtggagag ctggaaggac a 591

<210> 8
<211> 480
<212> DNA
<213> Eucalyptus grandis

<220>
<221> 5'UTR
<222> (1)...(480)

<400> 8
atgctgagag aaatgaattg ggaagtcgat cgacaatggc agctcaactc aatgatcctc 60
aggtataagc atttttttgg cagctctggt cattgtgtct tcaactttta gatgagagca 120
aatcaaattg actctaatac cagttatgtg atgagtgaat catttgcttt tagtagcttt 180
aatttatgcc cccatcttag ttgggtataa aggttcagag tgcgaagatt acatctattt 240
tggttcttgc aggacacagg gattcatgct agacacatca gcagtgtttc tacgttggat 300
agtggtatgt acttagctac tataaaggaa attttgatag atatgtttga tatggtgctt 360
gtacagatct atttaatgcc aatgtatttg aaactatctt gtctcataac tttcttgaag 420
aatacaatga tgagactggg aaccctatct ggaagaatag agtggagagc tggaaggaca 480

<210> 9
<211> 308

<212> DNA
<213> Eucalyptus grandis

<220>
<221> 5'UTR
<222> (1)...(259)

<400> 9
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gcccatctca ggtgcaacgg tttaactgat gtttactaca cgcaaggggg aggtatccgg      60
aaagcttgca aatcgggtaa aaacgaaaat gggcgacgtg gactcagcct gcccatgttt     120
tcggtctctc tcctggactt ccatgcccga taagggccgc caactctctc tctctctctc     180
tttttctctc acatctctct gcctgttcat gtcgcctgca agtgaagatt cgtcggagca     240
agaaggacga accgggcaca tggcggggtc ggcggtcgcg acggttctaa agggtctctt     300
cctggtgt                                                              308
```

<210> 10
<211> 300
<212> DNA
<213> Eucalyptus grandis

<220>
<221> 5'UTR
<222> (1)...(251)

<400> 10
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gcccatctca ggtgcaacgg tttaactgat gtttactaca cgcaaggggg aggtatccgg      60
aaagcttgca aatcgggtaa aaacgaaaat gggcgacgtg gactcagcct gcccatgttt     120
tcggtccctc tcctggactt ccatgcccga taaaggccgc caactctctc tctttttctc     180
tcacatctct ctgcctgttc atgtcgcctg caagtgaaga ttcgtcggag caagaaggac     240
gaactgggca tatggcgggg tcggcggtcg cgacggttct aaagggtctc ttcctggtgt     300
```

<210> 11
<211> 297
<212> DNA
<213> Eucalyptus grandis

<400> 11
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gtgcaacggt ttaactgatg tttactacac gcaaggggga ggtatccgga aagcttgcaa      60
atcgggtaaa aacgaaaatg ggcgacgtgg actcagcctg cccatgtttt cggtctctct     120
cctggacttc catgcccgat aagggccgcc aactctctct ctctctctct ttttctctca     180
catctctctg cctgttcatg tcgcctgcaa gtgaagattc gtcggagcaa gaaggacgaa     240
ctgggcatat ggcggggtcg gcggtcgcga cggttctaaa gggtctcttc ctggtgt        297
```

<210> 12
<211> 661
<212> DNA
<213> Eucalyptus grandis

<400> 12
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ctgagccatt taattcgaga gcacatcgcc caaaattatt cttcttgctg ccataactgt      60
cgaattttct cttttaggta agtaaccaat gatgcatcat gttgacaaaa aggctgatta     120
gtatgatctt ggagttgttg gtgcaaattt gcaagctgac gatggcccct cagggaaatt     180
aaggcgccaa cccagattgc aaagagcaca aagagcacga tccaaccttt ccttaacaag     240
atcatcacca gatcggccag taagggtaat attaatttaa caaatagctc ttgtaccggg     300
aactccgtat ttctctcact tccataaacc cctgattaat ttggtgggaa agcgacagcc     360
aacccacaaa aggtcagatg tcatcccacg agagagagag agagagagag agagagagag     420
agagttttct ctctatattc tggttcaccg gttggagtca atggcatgcg tgacgaatgt     480
```

acatattggt gtagggtcca atattttgcg ggagggttgg tgaaccgcaa agttcctata 540
tatcgaacct ccaccaccat acctcacttc aatccccacc atttatccgt tttatttcct 600
ctgctttcct ttgctcgagt ctcgcggaag agagagaaga gaggagagga gagaatgggt 660
t 661

<210> 13
<211> 336
<212> DNA
<213> Pinus radiata

<400> 13
actagtgatt tgttgagaat gagtaggcat tgctacaccc atcatcacaa gcatcatcat 60
gaggagaaga agatccattt ctcactctat tactcgaact tccttcagat taggctgtgt 120
atttctcact ctaccactcc aacttccttc aaatgctgtg agtttttgtt gtaattgccc 180
cgtctattta taatcgcagc agcactcgtc atataaagac ccgtgtgtgt gaacaacaac 240
caagtgattt gaattggaaa tgaagagcga gaatggcggt gtcatgaccg ggagcaacca 300
gcccgggccg tcgaccacgc gtgccctata gtaatc 336

<210> 14
<211> 763
<212> DNA
<213> Pinus radiata

<400> 14
actagtgatt tgttgagaat gagtaggcat tgctacaccc atcatcacaa gcatcaacat 60
gaagagaaga agacgatcca tttctcactc tatcactcca acttccttca gattaggctg 120
tgtatttctc actctaccac tccaactacc actccaactt attgccgcaa aagagagagg 180
ttcccaaact ctgtcggaat tctcccactc aaagcattaa aggaaagatc taattgctgc 240
aaaaaagaga gattcccaat atatttctca actcccttca aatgatttct cactctacca 300
ctccaactcc cttcaaatga tttctcactc taccactcca acttccttca aatgctgtga 360
gtttttgttg taattgcccc gtctatttat aatcgcagca gcactcgtca tataaagacc 420
cgtgcgtgtg aacaacaatg gcggtgtctt gactgggagc aaccgcataa agaaagtggg 480
cttcatacat taaaaaaatc tgtaaatttt acggatttgg aaaaaggaag agcaggaggg 540
acctcccgac ttgacccgag aatggcggtg tcttgaccgc gtaaagaaag tggtcttctg 600
tacccgactt gacccgaaaa aagaggaaac gttgaacgag acaatctctg ggaacttcat 660
cgaaatgaac ctcacgactt gactctttcg attgtactgt tttcattgtt cccgcgtaaa 720
acgaccagcc cgggccgtcg accacgcgtg ccctatagta atc 763

<210> 15
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 15
acggataaca gagtctttat attaaacgaa atggtattgc 40

<210> 16
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 16
tgacgcggcc gcgaccgacg aaaagaaaaa tataacataa gagagtctga a 51

<210> 17
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 17
tatagcggcc gcgggggggg ggggggg 27

<210> 18
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 18
cggagaacaa ggtggagggt agattctttc 30

<210> 19
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Made in a lab

<400> 19
tctgcatctt gaaattgaaa tatgactaac g 31

<210> 20
<211> 363
<212> DNA
<213> Eucalyptus grandis

<400> 20
aatcgggtga aaatagggcc gccctaaatt agaattgaca acatttcttg ggcaaagtta 60
atgtaagtta catgaaaaaa aaaaaaaagg atagtttgtt ggaagtaatg gagcatttgt 120
attgtgaaat tcacgataga gctaacaaaa ataaaggtag ttggtgggtt aacccagtta 180
aaaaagaaca ataatttgaa gagaggagag agagagagag gagggggaga gcatttcgat 240
aaattcacta gaaaaaatgc gtgttttagt ataaatgaga gtggaaatag ggccatctag 300
ggaacgatcg atcgcccctg cacccggcca tctggagagt ctgtttatac ttctctccgg 360
ctt 363

<210> 21
<211> 839
<212> DNA
<213> Pinus radiata

<220>
<221> misc_feature

<222> (1)...(839)
<223> n = A,T,C or G

<400> 21
gtatggagtt ttgaagggct ttactcttaa catttgtttt tctttgtaaa ttgttaatgg 60
tggtttctgt gggggaagaa tcttttgcca ggtccttttg ggtttcgcat gtttatttgg 120
gttatttttc tcgactatgg ctgacattac tagggctttc gtgctttcat ctgtgttttc 180
ttcccttaat aggtctgtct ctctggaata tttaatttc gtatgtaagt tatgagtagt 240
cgctgtttgt aataggctct tgtctgtaaa ggtttcagca ggtgtttgcg ttttattgcg 300
tcatgtgttt cagaaggcct ttgcagatta ttgcgttgta ctttaatatt ttgtctccaa 360
ccttgttata gtttccctcc tttgatctca caggaaccct ttcttctttg agcattttct 420
tgtggcgttc tgtagtaata ttttaatttt gggcccgggt tctgagggta ggtgattatt 480
cncagtgatg tgctttccct ataaggtcct ctatgtgtaa gctgttaggg tttgtgcgtt 540
actattgaca tgtcacatgt cacatatttt cttcctctta tccttcgaac tgatggttct 600
ttttctaatt cgtggattgc tggtgccata ttttatttct attgcaactg tattttaggg 660
tgtctctttc tttttgattt cttgttaata tttgtgttca ggttgtaact atgggttgct 720
agggtgtctg ccctcttctt ttgtgcttct ttcgcagaat ctgtccgttg gtctgtattt 780
gggtgatgaa ttatttattc cttgaagtat ctgtctaatt agcttgtgat gatgtgcag 839

<210> 22
<211> 881
<212> DNA
<213> Eucalyptus grandis

<400> 22
acgtgacgat gctcgagtct cgcgttctcc tctctcttgt tctgcaaaac agaaaagaga 60
gaatggaggt tggcctctct caattacgtg gacgccaatg agataactca ggtgggcgac 120
aaaacaaacg cctcttgatt tcctcaaacc ccaaaccgaa tccctcgtca aggggcaagg 180
cttttggtcc cgcggcccca cggatcgctc gttcccgtct cgccacgtcg cgtcgcagcg 240
tgtcgagcaa acagaggggt ccgagcgact ataaaatccc gacgccatcg acaccacagt 300
ccatcgaaaa ccttgttcaa ttcccaagtg aaagtgagta actgtgaacg aagagttgaa 360
ctttgcatct cggcgtgtgg attcaagagg aagcagcaaa gtggaaatgg acaactccaa 420
gatgggcttc aatgcagggc aggccaaggg ccagactcag gagaagagca accagatgat 480
ggataaggca tccaacactg ctcaatctgc aagggattcc atgcaagaga ctggtcagca 540
gatgaaggcc aaagcccagg gtgctgctga tgcagtgaag aatgccaccg ggatgaacaa 600
atgaagagct caagacatga atgaataaat aattaagctc tggttatcat ttgcttttcc 660
ggtcgtttgt tgtcctgttt ttccttgtca agagcttatt atgagggtcc ttttgctctt 720
tccttagttc tttttgtttc ttggttgttc catgaagaga gcaactctct gtgtttgaga 780
gtactcatct cgcttcataa ggtctcagta tgtagttgcc tttcgagaat gttatgttct 840
ctctcataat gctattctga ttttataaaa aaaaaaaaaa a 881

<210> 23
<211> 350
<212> DNA
<213> Eucalyptus grandis

<400> 23
ctatagggca cgcgtggtcg acggcccggg ctggtccttt cttacaaaaa gcaaaattct 60
tataattttt tttgatataa taaaaatgat ccataaactt ttgcttaatg tgcaacgtaa 120
accataatat attcaacgtg atgcttaaac tttaatcgag tatgcaatgt agtccataat 180
atattcaata tgatccttca atccaattga agtgtgcaat gtggtcgcta gattttttta 240
tgtattcaac ttagtcttta agctaccaac cttccaataa tttatgtttt agaaataata 300
tcgaacatct tttatattat tcaaggaata aaacgaacat gcatcaaaag 350

<210> 24
<211> 49
<212> DNA

<213> Eucalyptus grandis

<400> 24
actatagggc acgcgtggtc gacggcccgg gctggtactt ttttttct 49

<210> 25
<211> 909
<212> DNA
<213> Eucalyptus grandis

<400> 25
cagggtaaag aaaatggaat atttgcttgg ccccccagct ttgaaagttg ctgtaagaac 60
acactcacct tgcatttata cgatggttgt gagcagtgca ggctggtggt gctgcaaatt 120
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cagccgtgga 430

<210> 27
<211> 1253
<212> DNA
<213> Eucalyptus grandis

<400> 27
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<210> 28
<211> 99
<212> DNA
<213> Eucalyptus grandis

<400> 28

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<210> 29
<211> 927
<212> DNA
<213> Eucalyptus grandis

<400> 29

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gattggtcat gtcaccctca ccaaaggttg ccgaaggtcc ggtgacctca gctgacggcc      240
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<212> DNA
<213> Eucalyptus grandis

<400> 30

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<210> 31
<211> 178
<212> DNA
<213> Eucalyptus grandis

<400> 31
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aggaacttgg gaagaaccaa tgatgcctgg tcactgagtg atcgatgaat gcaatagt 178

<210> 32
<211> 178
<212> DNA
<213> Eucalyptus grandis

<400> 32
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<210> 33
<211> 178
<212> DNA
<213> Eucalyptus grandis

<400> 33
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<210> 34
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<212> DNA
<213> Eucalyptus grandis

<400> 34
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<212> DNA
<213> Eucalyptus grandis

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<212> DNA
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<400> 36
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<212> DNA
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<210> 38
<211> 288
<212> DNA
<213> Eucalyptus grandis

<400> 38
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<210> 39
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<212> DNA
<213> Eucalyptus grandis

<400> 39
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<210> 40
<211> 986
<212> DNA
<213> Eucalyptus grandis

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<210> 41
<211> 313
<212> DNA
<213> Pinus radiata

<400> 41
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<210> 42
<211> 713
<212> DNA
<213> Pinus radiata

<400> 42
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<210> 43
<211> 28
<212> DNA
<213> Pinus radiata

<400> 43
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<210> 44
<211> 35
<212> DNA
<213> Pinus radiata

<400> 44
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<210> 45
<211> 1729

<212> DNA
<213> Pinus radiata .

<400> 45
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tgaataatag atgtacagca aaattacagt agttgagaac aaagatggaa ggataatccc    1560
aacgatagct agcttggaca gtaggatgat tacatcaaaa tcatagcagt tgagaacata    1620
gttggaagga gaatccttat gatggctacg ttggataata ggcgtgatta tcgtaggtag    1680
attagagcac aagatcaaac taatagctgg cgcagctatc gactatttt                1729
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<210> 46
<211> 1038
<212> DNA
<213> Pinus radiata

<400> 46
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tcattcatta tataagatca gattcgtatg atatacaggc aaccatagaa acaaccagca     180
aagttactag caggaaatcc aactaggtat catgaagact accaacgcag gctcgataat     240
gttggtgctc attattttg ggtgctgttt cattggggtc atagctacat cttttgattt      300
ctattacttc gttcaacagt ggcctggttc atactgcgat actcgtagag gatgctgtta     360
ccctcgcacg ggaaggcctg cttccgaatt ttccattcat ggcctctggc ccaactacaa     420
gaccggtaaa tggccacagt tctgtggttc ctccgaagaa ttcgactact caaagatctc     480
agatctggag gaggagctga acaggtattg gggttcgtta agctgtccaa gcagcgatgg     540
acaggaattt tggggacacg agtgggagaa acatggcact tgctctctca atcttgatga     600
gcattcatac tttgagaagg ctctctcctt gagacaaaat atagacattc ttggggctct     660
taaaactgca ggtattaaac ccgatggaag ccaatacagt ttgagcgata tcaaggaagc     720
cattaaacaa aacactgggc agctcccagg aatcgattgc aacacgagcg cagagggaga     780
gcatcaacta tatcaggtgt atgtgtgtgt tgataaatcc gatgcttcca ctgttattga     840
atgccccatt tatccacaca gcaattgccc atccatggtt gtgtttcctc cttttgggga     900
ggatcaggag gaccgagatg gttacacaga aggaatgtac gagctgtaga tctggacaaa     960
cagcatttct tctctccgca tttgattttt atcaatgaaa tttccgattc caacattttg    1020
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taaaaaaaaa aaaaaaaa 1038

<210> 47
<211> 91
<212> DNA
<213> Pinus radiata

<400> 47
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tcctccgaag aattcgatat caagcttatc g 91

<210> 48
<211> 91
<212> DNA
<213> Pinus radiata

<400> 48
gcttttcatc cacactggtg cctcattcat tatataagat cagattcgtg tgatatacag 60
gcaaccatag aaacaaccgg caaagttact a 91

<210> 49
<211> 809
<212> DNA
<213> Pinus radiata

<400> 49
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catgaaaacc gattagggta ttgcaaatta gggcattgcc attcaaataa ttctcagatg 120
aaagattctc tctaacaatt acaaatgatt atttttttcc atgagtgttg catgttcgaa 180
cggtctgccc agtctgtgag agagcataga gaaccctccc tgcccaattt gttagagcat 240
agagaaccct actgcatgag tagtaagaaa aatattcggt ctcaattcgg caaagaccac 300
ctcgaatgga tgacttcaac gacaatctca tgatagtgtt ctgatcagca ccagttcacc 360
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tttcccagta tcatatatcc ttaatactgt tttgtctttt aatccatggc taccatcaga 480
acaagctcaa agcagaataa gggagcatca gccatcctct tgcttatcgc gattgcaggg 540
ttagtaaatg cgtgcaacgc tgtgggtatt gagccaatgt gcgacactgt ggtgtcgagt 600
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ccaagctgct gcaacgcggt tgagtcagct gggcttcaat gcctctgtct cgtcgttaac 720
ggccctcctt ttccaggggt cgaccgcggc ctcgcaatgc agctgcctgc caaatgccat 780
ctcacccttc ctccctgtaa cagttagtt 809

<210> 50
<211> 428
<212> DNA
<213> Eucalyptus grandis

<400> 50
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aggactaaag ttttccaccc aaatataaat aacaatggaa gtatctgcct tgacatcttg 120
aaggaacagt ggagtcctgc tttgacaatc tccaaggttt tgctctcaat ttgctctttg 180
ttgacggatc caaacccaga tgatcctctt gtaccagaga ttgctcatat gtacaagact 240
gataggggca aatatgagtc cactgcacgg agttggactc agaaatatgc aatgggttaa 300
ctttaaaaac tatatatcag tgatggaact ttatccctaa gttggaatct cttcgaatca 360
atgacttgtt tgcttgtaag aaatgtttcc ttaagataag tggctttcct caaaacttga 420
ttgaagtg 428

<210> 51

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<211> 525
<212> DNA
<213> Pinus radiata

<400> 51
cccttctttg ccttcaacta atcctgctca tcctctcctg cccccattcc caaagatggc      60
tgcacccaga tcatccgcta aattgggtgc acttttggca atactgctca tagttgcggc     120
agcgcaggct caagattgct caaatgccat ggacaaattg gctccatgca cttcagcagt     180
gggactgtct agcaatggag tgaagccctc atctgagtgc tgtgatgccc tcaaaggaac     240
cagtactggc tgcgtctgca agtctgtgag agcagtgata tcacttcctg ctaagtgcaa     300
tctcccagcc ataacctgct ctggatctcg ctgaaggctc tctgttatgg cgattctcag     360
atcgtggatc tctttaagat tttcagcaag caagtgatag aataaattct cagattttga     420
gatatctata tagcgatttt cagtatcaga ttgtctatag tactcatata tttaagtgat     480
tgaatagcat tctccgattc cgagttggaa acacagacac aatga                     525

<210> 52
<211> 1126
<212> DNA
<213> Pinus radiata

<400> 52
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atttaattgt tattgagcca gagagatgcg tagtcgctca tgtcacttgt gtttaccaaa     120
aagacataca taaacacctg cacctaaaag ttataatgat aacatgcata caaccctaca     180
acgtacgtag tcacatgcgg ctagaactta aacccctacc acaaacatag ccacctgcac     240
ccagaagtta taataataac atacatagaa cccttacaat aaaaaaagtt atctccaatg     300
attattaatc tactgcaggc cagccatact cagcttgaac gtgaaaattc gcattgtaag     360
catggcgcca cattaaaata acctcggcaa tattttcatg tccaagtggc cggccagcca     420
cgctcctcgc actctgagaa tactctattc atccacttgt ctctgccccg caactcatat     480
aaatgtggcc aacccaagca ccatatccat gttcattaat cccctctttg ccttcaacta     540
atcctgctca tcgcctcttg ccccaattcc caaagatggc tgcacccaga tcatccgcta     600
aatcggctgc acttttcgca atactgctca tagttgcggc agtacaggct gaagattgct     660
caaatgccat ggacaaattg gctccatgca cttcagcagt gggactgtct agcaatggag     720
tgaagccctc atctgagtgc tgtgatgccc tcaaaggaac cagtactggc tgcgtctgca     780
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ctggatctcg ctgaaggctc tctgttatgg cgattctcag atcgtggatc tctttaagat     900
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cagtatcaga ttgtctacag taccaatata tttaagtgat tgaatggaat tctcggattc    1020
tgagatagaa atataggcac agaatgtggc cggaggaatg ttcgaattcg agaatgataa    1080
taaataataa atgattgatt tctctctgca aaaaaaaaaa aaaaaa                  1126

<210> 53
<211> 454
<212> DNA
<213> Pinus radiata

<400> 53
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aattgggtgc acttttggca atactgctca tagttgcggc agcgcaggct caagattgct     120
caaatgccat ggacaaattg gctccatgca cttcagcagt gggactgtct agcaatggag     180
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agtctgtgag agcagtgata tcacttcctg ctaagtgcaa tctcccagcc ataacctgct     300
ctggatctcg ctgaaggctc tctgttatgg cgattctcag atcgtggata tctttaagat     360
tttcagcaag tgatagaata aattctcaga ttttgagata tctatatagc gattttcagt     420
atcagattgt ctatagtact catatattta agtg                                 454

<210> 54
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<211> 335
<212> DNA
<213> Pinus radiata

<400> 54
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tctgcaagga ttattgccct gtagttattt ggaagtagat cattttcaca ggcccagatg 120
cattatattc taatgcagtt gtttgttaat tgaagtgcaa atagttccaa aatgtttaca 180
tgaatcaata gtgaacaaat ccctctgttt tatatcatat tgatggatta ttcgattttt 240
tggtgacgtg gcgcgaaact gcttttcgaa ctcatggaaa tagtaattgt tataatccat 300
aggcatgaga ttcttgttaa tcgtgcacaa ggttt 335

<210> 55
<211> 336
<212> DNA
<213> Pinus radiata

<400> 55
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atgagttcga aaagcagttt cgcgccacgt caccaaaaaa tcgaataatc catcaatatg 120
atataaaaca gagggatttg ttcactattg attcatgtaa acattttgga actatttgca 180
cttcaattaa caaacaactg cattagaata taatgcatct ggtgcctgtg aaaatgatct 240
acttccaaat aactacaggg caataatcct tgcagactag ggcttatcta taagctcatg 300
aacaaagagc aggcctcctt tttaacaggt gcttct 336

<210> 56
<211> 532
<212> DNA
<213> Pinus radiata

<400> 56
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cgtttttggg gctcccttga agatctcctc ttcatttcgg gatttcctgc cttcgccgcg 120
ccatttgaag ttctttttct gagagaagaa tttagacatg gctgatcgca tgttgactcg 180
aagccacagc cttcgcgagc gtttggacga gaccctctct gctcaccgca acgatattgt 240
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tgaagtcctc aaatccactc aggaagcgat tgtgtcgcct ccatgggttg ctcttgctgt 420
tcgtccaagg ccgggcgtgt gggagcacat ccgtgtgaac gtccatgcgc ttgttcttga 480
gcaattggag gttgctgagt atctgcactt caaagaagag cttgctgatg ga 532

<210> 57
<211> 3103
<212> DNA
<213> Eucalyptus grandis

<400> 57
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tggtacactg aaacaacagg aaggtggtgg aggccgcaat gatggaattt atccacttta 120
atcattttat gaaatcgata cactaacctt tgtttctcct aaacccaaag gcattaatcc 180
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cgtgtataaa agggcctccc ccattcctca tttttcacca tcctccgttc gttcgttccc 300
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atggttcatt ttcttttcaa aaaaaaaaa aaaaaaaaa aaa 3103

<210> 58
<211> 326
<212> DNA
<213> Eucalyptus grandis

<400> 58
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ggatctgatt ggccgcgacg tccgcctctg acgtggcacc accgacgatt ttttttaat 180
atcttggtca agtcctaatt taactatggg gtccagatta gaagcttatc cactatggat 240
taaattaaat caaatgggaa ttaaattaaa ttaaaatcat cgtgcggagg tgcacgagat 300
gcacgagatc cgacggcgca gagcag 326

<210> 59

<211> 311
<212> DNA
<213> Eucalyptus grandis

<400> 59
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ttccaattta gccccttctg taattgctca tcttctttac caaattctct aatttggccg 120
gcgaagggct gacaagggat tggtcatgtc accctcacca aaggttgccg aaggtccggt 180
gacctcagct gacggccacc tacaccaaat ctagctcact agcagcctaa gcccttcatc 240
aactctagtg aaaggttttg agtatttttt aataaaaaat atttaaaaaa tatatagcga 300
gagctcatta c 311

<210> 60
<211> 2096
<212> DNA
<213> Eucalyptus grandis

<400> 60
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agtaaccaat gatgcatcat gttgacaaaa aggctgatta gtatgatctt ggagttgttg 180
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taagggtaat attaatttaa caaatagctc ttgtaccggg aactccgtat ttctctcact 360
tccataaacc cctgattaat ttggtgggaa agcgacagcc aacccacaaa aggtcagatg 420
tcatcccacg agagagagag agagagagag agagagagag agagttttct ctctatattc 480
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gatgaccccg acocaagtct cggacgagga ggcgaacctc ttcgccatgc agctggcgag 780
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cctctgtggt gatgttcatg gttcttggat ttgaaaggtc gtgaaggagc ccttttctca 1860
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gatggcgcaa gtggaagtta caagatttgt tgttttatgt ctataaagtt ttgagtcttc 1980
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<210> 61
<211> 522
<212> DNA

<213> Eucalyptus grandis

<400> 61
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ggagaagatt cacatcagca ttgtggtcat tggccatgtc gattctggga agtcaaccac 180
aactggccac ttgatataca agctcggagg aatcgacaag cgtgtgattg agagattcga 240
gaaggaagct gctgagatga acaagagatc gttcaagtat gcttgggtgc ttgacaagct 300
caaggccgag cgcgagcgcg gtattaccat tgatattgcc ttgtggaagt tcgagaccac 360
caagtactac tgcactgtca ttgatgctcc tggacatcgt gactttatta agaatatgat 420
tactggaacc tcccaggccg actgtgctgt ccttatcatt gattccacca ctggtggttt 480
cgaagctggt atttccaagg atggccagac ccgtgaacat gc 522

<210> 62
<211> 420
<212> DNA
<213> Eucalyptus grandis

<400> 62
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agggcaattc tgtttctcta gtgtaaataa gggtgtattt aataattgag ggatggaaat 180
agcatggtca ctcggtaatt atcaaggaaa gcaagaataa aaatggaaaa aaaaaaaaaa 240
aaagcttgaa gaggccaatg tcgaaattat gagcgcgaga tgaggacact cctgggaaac 300
gaaaaatggc attcgcgggg ggtgctatat aaagcctcgt gtaagggtgc gttcctcact 360
ctcaaaccct aatcctgccc ttcccttctg ctgctgctgc tcgtcacctc tctcctccct 420

<210> 63
<211> 65
<212> PRT
<213> Eucalyptus grandis

<400> 63
Met Asp Asn Ser Lys Met Gly Phe Asn Ala Gly Gln Ala Lys Gly Gln
1 5 10 15
Thr Gln Glu Lys Ser Asn Gln Met Met Asp Lys Ala Ser Asn Thr Ala
20 25 30
Gln Ser Ala Arg Asp Ser Met Gln Glu Thr Gly Gln Gln Met Lys Ala
35 40 45
Lys Ala Gln Gly Ala Ala Asp Ala Val Lys Asn Ala Thr Gly Met Asn
50 55 60
Lys
65

<210> 64
<211> 152
<212> PRT
<213> Eucalyptus grandis

<400> 64
Met Gly Gly Pro Leu Thr Leu Asp Ala Glu Val Glu Val Lys Ser Pro
1 5 10 15
Ala Asp Lys Phe Trp Val Ser Val Arg Asp Ser Thr Lys Leu Phe Pro
20 25 30
Lys Ile Phe Pro Asp Gln Tyr Lys Asn Ile Glu Val Leu Glu Gly Asp
35 40 45
Gly Lys Ala Pro Gly Ser Val Arg Leu Phe Thr Tyr Gly Glu Gly Ser

```
    50                  55                  60
Pro Leu Val Lys Val Ser Lys Glu Lys Ile Asp Gly Val Asp Glu Ala
65                  70                  75                  80
Asp Lys Val Val Thr Tyr Ser Val Ile Asp Gly Asp Leu Leu Lys Tyr
                85                  90                  95
Tyr Lys Asn Phe Asn Gly Ser Ile Lys Val Ile Pro Lys Gly Asp Gly
            100                 105                 110
Ser Leu Val Lys Trp Ser Cys Gly Phe Glu Lys Ala Ser Asp Glu Ile
        115                 120                 125
Pro Asp Pro His Val Ile Lys Asp Phe Ala Ile Gln Asn Phe Lys Glu
    130                 135                 140
Leu Asp Glu Phe Ile Leu Lys Ala
145                 150

<210> 65
<211> 117
<212> PRT
<213> Eucalyptus grandis

<400> 65
Met Ala Ala Asn Phe Val Ile Pro Thr Lys Met Lys Ala Trp Val Tyr
 1               5                  10                  15
Arg Glu His Gly Asn Val Ala Asp Val Leu Gly Leu Asp Pro Glu Leu
            20                  25                  30
Lys Val Pro Glu Leu Gln Glu Gly Gln Val Leu Val Lys Val Leu Ala
        35                  40                  45
Ala Ala Leu Asn Pro Val Asp Ala Ala Arg Met Lys Gly Val Ile Lys
    50                  55                  60
Leu Pro Gly Phe Ser Leu Pro Ala Val Pro Gly Tyr Asp Leu Ala Gly
65                  70                  75                  80
Val Val Val Lys Val Gly Arg Glu Val Lys Glu Leu Lys Ile Gly Asp
                85                  90                  95
Glu Val Tyr Gly Phe Met Phe His Ala Lys Lys Asp Gly Thr Leu Ala
            100                 105                 110
Glu Tyr Ala Ala Val
        115

<210> 66
<211> 318
<212> PRT
<213> Eucalyptus grandis

<400> 66
Met Ala Ala Asn Phe Val Ile Pro Thr Lys Met Lys Ala Trp Val Tyr
 1               5                  10                  15
Arg Glu His Gly Asp Val Ala Asn Val Leu Gly Leu Asp Pro Glu Leu
            20                  25                  30
Lys Val Pro Glu Leu Gln Glu Gly Gln Val Leu Val Lys Val Leu Ala
        35                  40                  45
Ala Ala Leu Asn Pro Ile Asp Thr Ala Arg Val Lys Gly Val Ile Lys
    50                  55                  60
Leu Pro Gly Phe Ser Leu Pro Ala Val Pro Gly Tyr Asp Leu Ala Gly
65                  70                  75                  80
Val Val Val Lys Val Gly Arg Glu Val Lys Glu Leu Lys Val Gly Asp
                85                  90                  95
Glu Val Tyr Gly Phe Met Phe His Ala Lys Lys Asp Gly Thr Leu Ala
            100                 105                 110
```

```
Glu Tyr Ala Ala Val Glu Glu Ser Phe Leu Ala Leu Lys Pro Lys Lys
        115                 120                 125
Leu Arg Phe Gly Glu Ala Ala Ser Leu Pro Val Val Ile Gln Thr Ala
    130                 135                 140
Tyr Gly Gly Leu Glu Arg Ala Gly Leu Ser His Gly Lys Ser Leu Leu
145                 150                 155                 160
Val Leu Gly Gly Ala Gly Gly Val Gly Thr Leu Ile Ile Gln Leu Ala
                165                 170                 175
Lys Glu Val Phe Gly Ala Ser Arg Val Ala Ala Thr Ser Ser Thr Gly
            180                 185                 190
Lys Leu Glu Leu Leu Lys Ser Leu Gly Ala Asp Leu Ala Ile Asp Tyr
        195                 200                 205
Thr Lys Val Asn Phe Glu Asp Leu Pro Glu Lys Phe Asp Val Val Tyr
    210                 215                 220
Asp Thr Val Gly Glu Ile Glu Arg Ala Ala Lys Ala Val Lys Pro Gly
225                 230                 235                 240
Gly Ser Ile Val Thr Ile Val Lys Gln Asn Lys Thr Leu Pro Pro Pro
                245                 250                 255
Ala Phe Phe Phe Ala Val Thr Ser Asn Arg Ser Thr Leu Glu Lys Leu
            260                 265                 270
Lys Pro Phe Leu Glu Ser Gly Lys Val Lys Pro Val Ile Asp Pro Lys
        275                 280                 285
Ser Pro Phe Pro Phe Ser Gln Ala Ile Glu Ala Phe Ser Tyr Leu Gln
    290                 295                 300
Thr Arg Arg Ala Thr Gly Lys Leu Val Ile His Pro Val Pro
305                 310                 315

<210> 67
<211> 156
<212> PRT
<213> Eucalyptus grandis

<400> 67
Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu
 1               5                  10                  15
Val Glu Ser Ser Asp Thr Val Asp Asn Val Lys Ala Lys Ile Gln Asp
            20                  25                  30
Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys
        35                  40                  45
Gln Leu Glu Asp Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu
    50                  55                  60
Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Met Gln Ile Phe
65                  70                  75                  80
Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu Val Glu Ser Ser
                85                  90                  95
Asp Thr Val Asp Asn Val Lys Ala Lys Ile Gln Asp Lys Glu Gly Ile
            100                 105                 110
Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp
        115                 120                 125
Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu Ser Thr Leu His
    130                 135                 140
Leu Val Leu Arg Leu Lys Gly Gly Met Gln Ile Phe
145                 150                 155

<210> 68
<211> 238
<212> PRT
```

<213> Eucalyptus grandis

<400> 68
```
Met Ala Thr His Ala Ala Leu Ala Pro Ser Thr Leu Pro Ala Asn Ala
1               5                   10                  15
Lys Phe Ser Ser Lys Ser Ser Ser His Ser Phe Pro Thr Gln Cys Phe
            20                  25                  30
Ser Lys Arg Leu Glu Val Ala Glu Phe Ser Gly Leu Arg Ala Gly Ser
        35                  40                  45
Cys Val Thr Tyr Ala Lys Asn Ala Gly Glu Gly Ser Phe Phe Asp Ala
    50                  55                  60
Val Ala Ala Gln Leu Thr Pro Lys Thr Ser Ala Pro Ala Pro Ala Lys
65                  70                  75                  80
Gly Glu Thr Val Ala Lys Leu Lys Val Ala Ile Asn Gly Phe Gly Arg
                85                  90                  95
Ile Gly Arg Asn Phe Leu Arg Cys Trp His Gly Arg Lys Asn Ser Pro
            100                 105                 110
Leu Asp Val Ile Val Val Asn Asp Ser Gly Gly Val Lys Asn Ala Ser
        115                 120                 125
His Leu Leu Lys Tyr Asp Ser Met Leu Gly Thr Phe Lys Ala Asp Val
    130                 135                 140
Lys Ile Val Asp Asn Glu Thr Ile Ser Val Asp Gly Lys Pro Val Lys
145                 150                 155                 160
Val Val Ser Asn Arg Asp Pro Leu Lys Leu Pro Trp Ala Glu Leu Gly
                165                 170                 175
Ile Asp Ile Val Ile Glu Gly Thr Gly Val Phe Val Asp Gly Pro Gly
            180                 185                 190
Ala Gly Lys His Ile Gln Ala Gly Ala Lys Lys Val Ile Ile Thr Ala
        195                 200                 205
Pro Ala Lys Gly Ala Asp Ile Pro Thr Tyr Val Tyr Gly Val Asn Glu
    210                 215                 220
Thr Asp Tyr Ser His Glu Val Ala Asn Ile Ile Ser Asn Ala
225                 230                 235
```

<210> 69
<211> 168
<212> PRT
<213> Eucalyptus grandis

<400> 69
```
Met Ser Thr Ser Pro Val Ser Ser Trp Cys Ala Thr Ser Phe Ser Pro
1               5                   10                  15
Ala His Ser Ser Leu Lys Arg Ala Ala Gly Leu Arg Pro Ser Leu Ser
            20                  25                  30
Ala Arg Leu Gly Pro Ser Ser Ser Ser Ser Ser Val Ser Pro Pro Thr
        35                  40                  45
Leu Ile Arg Asn Glu Pro Val Phe Ala Ala Pro Ala Pro Val Ile Asn
    50                  55                  60
Pro Thr Trp Thr Glu Glu Met Gly Lys Asp Tyr Asp Glu Ala Ile Glu
65                  70                  75                  80
Ala Leu Lys Lys Leu Leu Ser Glu Lys Gly Asp Leu Lys Ala Thr Ala
                85                  90                  95
Ala Ala Lys Val Glu Gln Ile Thr Ala Glu Leu Gln Thr Ala Ser Pro
            100                 105                 110
Asp Ile Lys Pro Ser Ser Ser Val Asp Arg Ile Lys Thr Gly Phe Thr
        115                 120                 125
Phe Phe Lys Lys Glu Lys Tyr Asp Lys Asn Pro Ala Leu Tyr Gly Glu
```

```
    130                 135                 140
Leu Ala Lys Gln Ser Pro Lys Phe Met Val Phe Ala Cys Ser Asp Ser
145                 150                 155                 160
Arg Val Cys Pro Ser His Val Leu
                165

<210> 70
<211> 214
<212> PRT
<213> Eucalyptus grandis

<400> 70
Met Pro Cys Pro Arg Ala Pro Pro Met Met Glu Arg Arg Ile Lys Pro
 1               5                  10                  15
Gln Thr Glu Gln Ala Leu Lys Cys Pro Arg Cys Asp Ser Thr Asn Thr
            20                  25                  30
Lys Phe Cys Tyr Tyr Asn Asn Tyr Asn Leu Ser Gln Pro Arg His Phe
        35                  40                  45
Cys Lys Thr Cys Arg Arg Tyr Trp Thr Lys Gly Gly Ala Leu Arg Asn
    50                  55                  60
Val Pro Val Gly Gly Gly Cys Arg Lys Asn Lys Arg Ala Lys Arg Ala
65                  70                  75                  80
Val Asp His Pro Val Ser Ala Gln Asn Glu Ala Ser Thr Ser Ala Ala
                85                  90                  95
Pro Gly Asn Glu Val Pro Asp Arg Ser Pro Phe Glu Pro Pro Ser Ser
            100                 105                 110
Lys Ser Ile Tyr Tyr Gly Gly Glu Asn Met Asn Leu Thr Gly Leu Pro
        115                 120                 125
Phe Ser Arg Ile Gln Gln Asp Arg Ala Ala Leu Ala His Cys Asn Ser
    130                 135                 140
Ser Ser Phe Leu Gly Met Ser Cys Gly Thr Gln Ser Ala Ser Leu Glu
145                 150                 155                 160
Pro His Leu Ser Ala Leu Asn Thr Phe Asn Ser Phe Lys Ser Asn Asn
                165                 170                 175
Pro Gly Leu Asp Phe Pro Ser Leu Ser Thr Asp Gln Asn Ser Leu Phe
            180                 185                 190
Glu Thr Ser Gln Pro Gln Leu Ser Arg Ala Met Ala Ser Ala Leu Phe
        195                 200                 205
Ser Met Pro Met Ala Pro
    210

<210> 71
<211> 166
<212> PRT
<213> Pinus radiata

<400> 71
Met Ala Ala Leu Ala Thr Thr Glu Val Cys Asp Thr Tyr Pro Arg Leu
 1               5                  10                  15
Val Glu Asn Gly Glu Leu Arg Val Leu Gln Pro Ile Phe Gln Ile Tyr
            20                  25                  30
Gly Arg Arg Arg Ala Phe Ser Gly Pro Ile Val Thr Leu Lys Val Phe
        35                  40                  45
Glu Asp Asn Val Leu Leu Arg Glu Phe Leu Glu Glu Arg Gly Asn Gly
    50                  55                  60
Arg Val Leu Val Val Asp Gly Gly Gly Ser Leu Arg Cys Ala Ile Leu
65                  70                  75                  80
```

```
Gly Gly Asn Val Val Val Ser Ala Gln Asn Asn Gly Trp Ser Gly Ile
                85                  90                  95
Ile Val Thr Gly Cys Ile Arg Asp Val Asp Glu Ile Asn Arg Cys Asp
            100                 105                 110
Ile Gly Ile Arg Ala Leu Thr Ser Asn Pro Leu Lys Ala Asn Lys Lys
        115                 120                 125
Gly Val Gly Glu Lys His Ala Pro Ile Tyr Ile Ala Gly Thr Arg Ile
    130                 135                 140
Leu Pro Gly Glu Trp Cys Tyr Ala Asp Ser Asp Gly Ile Leu Val Ser
145                 150                 155                 160
Gln Gln Glu Leu Ser Leu
                165

<210> 72
<211> 236
<212> PRT
<213> Pinus radiata

<400> 72
Met Leu Val Leu Ile Ile Phe Gly Cys Cys Phe Ile Gly Val Ile Ala
 1               5                  10                  15
Thr Ser Phe Asp Phe Tyr Tyr Phe Val Gln Gln Trp Pro Gly Ser Tyr
            20                  25                  30
Cys Asp Thr Arg Arg Gly Cys Cys Tyr Pro Arg Thr Gly Arg Pro Ala
        35                  40                  45
Ser Glu Phe Ser Ile His Gly Leu Trp Pro Asn Tyr Lys Thr Gly Lys
    50                  55                  60
Trp Pro Gln Phe Cys Gly Ser Ser Glu Glu Phe Asp Tyr Ser Lys Ile
65                  70                  75                  80
Ser Asp Leu Glu Glu Glu Leu Asn Arg Tyr Trp Gly Ser Leu Ser Cys
                85                  90                  95
Pro Ser Ser Asp Gly Gln Glu Phe Trp Gly His Glu Trp Glu Lys His
            100                 105                 110
Gly Thr Cys Ser Leu Asn Leu Asp Glu His Ser Tyr Phe Glu Lys Ala
        115                 120                 125
Leu Ser Leu Arg Gln Asn Ile Asp Ile Leu Gly Ala Leu Lys Thr Ala
    130                 135                 140
Gly Ile Lys Pro Asp Gly Ser Gln Tyr Ser Leu Ser Asp Ile Lys Glu
145                 150                 155                 160
Ala Ile Lys Gln Asn Thr Gly Gln Leu Pro Gly Ile Asp Cys Asn Thr
                165                 170                 175
Ser Ala Glu Gly Glu His Gln Leu Tyr Gln Val Tyr Val Cys Val Asp
            180                 185                 190
Lys Ser Asp Ala Ser Thr Val Ile Glu Cys Pro Ile Tyr Pro His Ser
        195                 200                 205
Asn Cys Pro Ser Met Val Val Phe Pro Pro Phe Gly Glu Asp Gln Glu
    210                 215                 220
Asp Arg Asp Gly Tyr Thr Glu Gly Met Tyr Glu Leu
225                 230                 235

<210> 73
<211> 92
<212> PRT
<213> Pinus radiata

<400> 73
Met Ala Ala Pro Arg Ser Ser Ala Lys Leu Gly Ala Leu Leu Ala Ile
```

```
 1               5                   10                  15
Leu Leu Ile Val Ala Ala Ala Gln Ala Gln Asp Cys Ser Asn Ala Met
            20                  25                  30
Asp Lys Leu Ala Pro Cys Thr Ser Ala Val Gly Leu Ser Ser Asn Gly
        35                  40                  45
Val Lys Pro Ser Ser Glu Cys Cys Asp Ala Leu Lys Gly Thr Ser Thr
    50                  55                  60
Gly Cys Val Cys Lys Ser Val Arg Ala Val Ile Ser Leu Pro Ala Lys
65                  70                  75                  80
Cys Asn Leu Pro Ala Ile Thr Cys Ser Gly Ser Arg
                85                  90
```

<210> 74
<211> 92
<212> PRT
<213> Pinus radiata

<400> 74

```
Met Ala Ala Pro Arg Ser Ser Ala Lys Ser Ala Ala Leu Phe Ala Ile
 1               5                   10                  15
Leu Leu Ile Val Ala Ala Val Gln Ala Glu Asp Cys Ser Asn Ala Met
            20                  25                  30
Asp Lys Leu Ala Pro Cys Thr Ser Ala Val Gly Leu Ser Ser Asn Gly
        35                  40                  45
Val Lys Pro Ser Ser Glu Cys Cys Asp Ala Leu Lys Gly Thr Ser Thr
    50                  55                  60
Gly Cys Val Cys Lys Ser Val Arg Ala Val Ile Ser Leu Pro Ala Lys
65                  70                  75                  80
Cys Asn Leu Pro Ala Leu Thr Cys Ser Gly Ser Arg
                85                  90
```

<210> 75
<211> 92
<212> PRT
<213> Pinus radiata

<400> 75

```
Met Ala Ala Pro Arg Ser Ser Ala Lys Leu Gly Ala Leu Leu Ala Ile
 1               5                   10                  15
Leu Leu Ile Val Ala Ala Ala Gln Ala Gln Asp Cys Ser Asn Ala Met
            20                  25                  30
Asp Lys Leu Ala Pro Cys Thr Ser Ala Val Gly Leu Ser Ser Asn Gly
        35                  40                  45
Val Lys Pro Ser Ser Glu Cys Cys Asp Ala Leu Lys Gly Thr Ser Thr
    50                  55                  60
Gly Cys Val Cys Lys Ser Val Arg Ala Val Ile Ser Leu Pro Ala Lys
65                  70                  75                  80
Cys Asn Leu Pro Ala Ile Thr Cys Ser Gly Ser Arg
                85                  90
```

<210> 76
<211> 125
<212> PRT
<213> Eucalyptus grandis

<400> 76

```
Met Ala Asp Arg Met Leu Thr Arg Ser His Ser Leu Arg Glu Arg Leu
```

```
 1               5                   10                  15
Asp Glu Thr Leu Ser Ala His Arg Asn Asp Ile Val Ala Phe Leu Ser
            20                  25                  30
Arg Val Glu Ala Lys Gly Lys Gly Ile Leu Gln Arg His Gln Ile Phe
        35                  40                  45
Ala Glu Phe Glu Ala Ile Ser Glu Glu Ser Arg Ala Lys Leu Leu Asp
    50                  55                  60
Gly Ala Phe Gly Glu Val Leu Lys Ser Thr Gln Glu Ala Ile Val Ser
65                  70                  75                  80
Pro Pro Trp Val Ala Leu Ala Val Arg Pro Arg Pro Gly Val Trp Glu
                85                  90                  95
His Ile Arg Val Asn Val His Ala Leu Val Leu Glu Gln Leu Glu Val
            100                 105                 110
Ala Glu Tyr Leu His Phe Lys Glu Glu Leu Ala Asp Gly
        115                 120                 125
```

<210> 77
<211> 805
<212> PRT
<213> Eucalyptus grandis

<400> 77

```
Met Ala Asp Arg Met Leu Thr Arg Ser His Ser Leu Arg Glu Arg Leu
 1               5                   10                  15
Asp Glu Thr Leu Ser Ala His Arg Asn Asp Ile Val Ala Phe Leu Ser
            20                  25                  30
Arg Val Glu Ala Lys Gly Lys Gly Ile Leu Gln Arg His Gln Ile Phe
        35                  40                  45
Ala Glu Phe Glu Ala Ile Ser Glu Glu Ser Arg Ala Lys Leu Leu Asp
    50                  55                  60
Gly Ala Phe Gly Glu Val Leu Lys Ser Thr Gln Glu Ala Ile Val Ser
65                  70                  75                  80
Pro Pro Trp Val Ala Leu Ala Val Arg Pro Arg Pro Gly Val Trp Glu
                85                  90                  95
His Ile Arg Val Asn Val His Ala Leu Val Leu Glu Gln Leu Glu Val
            100                 105                 110
Ala Glu Tyr Leu His Phe Lys Glu Glu Leu Ala Asp Gly Ser Leu Asn
        115                 120                 125
Gly Asn Phe Val Leu Glu Leu Asp Phe Glu Pro Phe Thr Ala Ser Phe
    130                 135                 140
Pro Arg Pro Thr Leu Ser Lys Ser Ile Gly Asn Gly Val Glu Phe Leu
145                 150                 155                 160
Asn Arg His Leu Ser Ala Lys Leu Phe His Asp Lys Glu Ser Leu His
                165                 170                 175
Pro Leu Leu Glu Phe Leu Gln Val His Cys Tyr Lys Gly Lys Asn Met
            180                 185                 190
Met Val Asn Ala Arg Ile Gln Asn Val Phe Ser Leu Gln His Val Leu
        195                 200                 205
Arg Lys Ala Glu Glu Tyr Leu Thr Ser Leu Lys Pro Glu Thr Pro Tyr
    210                 215                 220
Ser Gln Phe Glu His Lys Phe Gln Glu Ile Gly Leu Glu Arg Gly Trp
225                 230                 235                 240
Gly Asp Thr Ala Glu Arg Val Leu Glu Met Ile Gln Leu Leu Leu Asp
                245                 250                 255
Leu Leu Glu Ala Pro Asp Pro Cys Thr Leu Glu Lys Phe Leu Asp Arg
            260                 265                 270
Val Pro Met Val Phe Asn Val Val Ile Met Ser Pro His Gly Tyr Phe
```

```
        275                     280                     285
Ala Gln Asp Asp Val Leu Gly Tyr Pro Asp Thr Gly Gly Gln Val Val
    290                     295                     300
Tyr Ile Leu Asp Gln Val Arg Ala Leu Glu Glu Glu Met Leu His Arg
305                 310                     315                 320
Ile Lys Gln Gln Gly Leu Asp Ile Thr Pro Arg Ile Leu Ile Ile Thr
                325                     330                     335
Arg Leu Leu Pro Asp Ala Val Gly Thr Thr Cys Gly Gln Arg Leu Glu
            340                     345                     350
Lys Val Phe Gly Thr Glu Tyr Ser His Ile Leu Arg Val Pro Phe Arg
        355                     360                     365
Asn Glu Lys Gly Val Val Arg Lys Trp Ile Ser Arg Phe Glu Val Trp
    370                     375                     380
Pro Tyr Leu Glu Arg Tyr Thr Glu Asp Val Ala Ser Glu Leu Ala Gly
385                 390                     395                 400
Glu Leu Gln Gly Lys Pro Asp Leu Ile Ile Gly Asn Tyr Ser Asp Gly
                405                     410                     415
Asn Ile Val Ala Ser Leu Leu Ala His Lys Leu Gly Val Thr Gln Cys
            420                     425                     430
Thr Ile Ala His Ala Leu Glu Lys Thr Lys Tyr Pro Glu Ser Asp Ile
        435                     440                     445
Tyr Trp Lys Lys Phe Glu Glu Lys Tyr His Phe Ser Cys Gln Phe Thr
    450                     455                     460
Ala Asp Leu Ile Ala Met Asn His Thr Asp Phe Ile Ile Thr Ser Thr
465                 470                     475                 480
Phe Gln Glu Ile Ala Gly Ser Lys Asp Thr Val Gly Gln Tyr Glu Ser
                485                     490                     495
His Met Asn Phe Thr Leu Pro Gly Leu Tyr Arg Val Val His Gly Ile
            500                     505                     510
Asp Val Phe Asp Pro Lys Phe Asn Ile Val Ser Pro Gly Ala Asp Met
        515                     520                     525
Ser Ile Tyr Phe Ala Tyr Thr Glu Gln Glu Arg Arg Leu Lys Ser Phe
    530                     535                     540
His Pro Glu Ile Glu Glu Leu Leu Phe Ser Asp Val Glu Asn Lys Glu
545                 550                     555                 560
His Leu Cys Val Leu Lys Asp Lys Lys Lys Pro Ile Ile Phe Thr Met
                565                     570                     575
Ala Arg Leu Asp Arg Val Lys Asn Leu Thr Gly Leu Val Glu Trp Tyr
            580                     585                     590
Gly Lys Asn Ser Lys Leu Arg Glu Leu Ala Asn Leu Val Val Val Gly
        595                     600                     605
Gly Asp Arg Arg Lys Asp Ser Lys Asp Leu Glu Glu Gln Ser Glu Met
    610                     615                     620
Lys Lys Met Tyr Asp Leu Ile Glu Lys Tyr Lys Leu Asn Gly Gln Phe
625                 630                     635                 640
Arg Trp Ile Ser Ser Gln Met Asn Arg Val Arg Asn Gly Glu Leu Tyr
                645                     650                     655
Arg Tyr Ile Cys Asp Thr Lys Gly Val Phe Val Gln Pro Ala Ile Tyr
            660                     665                     670
Glu Ala Phe Gly Leu Thr Val Val Glu Ala Met Thr Cys Gly Leu Pro
        675                     680                     685
Thr Phe Ala Thr Cys Asn Gly Gly Pro Ala Glu Ile Ile Val His Gly
    690                     695                     700
Lys Ser Gly Tyr His Ile Asp Pro Tyr His Gly Asp Gln Ala Ala Glu
705                 710                     715                 720
Leu Leu Val Asp Phe Phe Asn Lys Cys Lys Ile Asp Gln Ser His Trp
                725                     730                     735
```

```
Asp Glu Ile Ser Lys Gly Ala Met Gln Arg Ile Glu Glu Lys Tyr Thr
            740                 745                 750
Trp Lys Ile Tyr Ser Glu Arg Leu Leu Asn Leu Thr Ala Val Tyr Gly
        755                 760                 765
Phe Trp Lys His Val Thr Asn Leu Asp Arg Arg Glu Ser Arg Arg Tyr
    770                 775                 780
Leu Glu Met Phe Tyr Ala Leu Lys Tyr Arg Pro Leu Ala Gln Ser Val
785                 790                 795                 800
Pro Pro Ala Val Glu
                805
```

<210> 78
<211> 264
<212> PRT
<213> Eucalyptus grandis

<400> 78

```
Met Gly Ser Thr Gly Ser Glu Thr Gln Met Thr Pro Thr Gln Val Ser
 1               5                  10                  15
Asp Glu Glu Ala Asn Leu Phe Ala Met Gln Leu Ala Ser Ala Ser Val
            20                  25                  30
Leu Pro Met Val Leu Lys Ala Ala Ile Glu Leu Asp Leu Leu Glu Ile
        35                  40                  45
Met Ala Lys Ala Gly Pro Gly Ala Phe Leu Ser Pro Gly Glu Val Ala
    50                  55                  60
Ala Gln Leu Pro Thr Gln Asn Pro Glu Ala Pro Val Met Leu Asp Arg
65                  70                  75                  80
Ile Phe Arg Leu Leu Ala Ser Tyr Ser Val Leu Thr Cys Thr Leu Arg
                85                  90                  95
Asp Leu Pro Asp Gly Lys Val Glu Arg Leu Tyr Gly Leu Ala Pro Val
            100                 105                 110
Cys Lys Phe Leu Val Lys Asn Glu Asp Gly Val Ser Ile Ala Ala Leu
        115                 120                 125
Asn Leu Met Asn Gln Asp Lys Ile Leu Met Glu Ser Trp Tyr Tyr Leu
    130                 135                 140
Lys Asp Ala Val Leu Glu Gly Gly Ile Pro Phe Asn Lys Ala Tyr Gly
145                 150                 155                 160
Met Thr Ala Phe Glu Tyr His Gly Thr Asp Pro Arg Phe Asn Lys Ile
                165                 170                 175
Phe Asn Arg Gly Met Ser Asp His Ser Thr Ile Thr Met Lys Lys Ile
            180                 185                 190
Leu Glu Thr Tyr Lys Gly Phe Glu Gly Leu Glu Thr Val Val Asp Val
        195                 200                 205
Gly Gly Gly Thr Gly Ala Val Leu Ser Met Ile Val Ala Lys Tyr Pro
    210                 215                 220
Ser Met Lys Gly Ile Asn Phe Asp Arg Pro Asn Gly Leu Lys Thr Pro
225                 230                 235                 240
His Pro Phe Leu Val Ser Ser Thr Ser Glu Ala Thr Cys Ser Ser Ala
                245                 250                 255
Phe Gln Arg Glu Met Pro Phe Ser
            260
```

<210> 79
<211> 136
<212> PRT
<213> Eucalyptus grandis

<400> 79
Met Gly Lys Glu Lys Ile His Ile Ser Ile Val Val Ile Gly His Val
1 5 10 15
Asp Ser Gly Lys Ser Thr Thr Thr Gly His Leu Ile Tyr Lys Leu Gly
20 25 30
Gly Ile Asp Lys Arg Val Ile Glu Arg Phe Glu Lys Glu Ala Ala Glu
35 40 45
Met Asn Lys Arg Ser Phe Lys Tyr Ala Trp Val Leu Asp Lys Leu Lys
50 55 60
Ala Glu Arg Glu Arg Gly Ile Thr Ile Asp Ile Ala Leu Trp Lys Phe
65 70 75 80
Glu Thr Thr Lys Tyr Tyr Cys Thr Val Ile Asp Ala Pro Gly His Arg
85 90 95
Asp Phe Ile Lys Asn Met Ile Thr Gly Thr Ser Gln Ala Asp Cys Ala
100 105 110
Val Leu Ile Ile Asp Ser Thr Thr Gly Gly Phe Glu Ala Gly Ile Ser
115 120 125
Lys Asp Gly Gln Thr Arg Glu His
130 135

<210> 80
<211> 229
<212> PRT
<213> Eucalyptus grandis

<400> 80
Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu
1 5 10 15
Val Glu Ser Ser Asp Thr Ile Asp Asn Val Lys Ala Lys Ile Gln Asp
20 25 30
Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys
35 40 45
Gln Leu Glu Asp Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu
50 55 60
Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly Met Gln Ile Phe
65 70 75 80
Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu Val Glu Ser Ser
85 90 95
Asp Thr Ile Asp Asn Val Lys Ala Lys Ile Gln Asp Lys Glu Gly Ile
100 105 110
Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp
115 120 125
Gly Arg Thr Leu Ala Asp Tyr Asn Ile Gln Lys Glu Ser Thr Leu His
130 135 140
Leu Val Leu Arg Leu Arg Gly Gly Met Gln Ile Phe Val Lys Thr Leu
145 150 155 160
Thr Gly Lys Thr Ile Thr Leu Glu Val Glu Ser Ser Asp Thr Ile Asp
165 170 175
Asn Val Lys Ala Lys Ile Gln Asp Lys Glu Gly Ile Pro Pro Asp Gln
180 185 190
Gln Arg Leu Ile Phe Ala Gly Lys Gln Leu Glu Asp Gly Arg Thr Leu
195 200 205
Ala Asp Tyr Asn Ile Gln Lys Glu Ser Thr Leu His Leu Val Leu Arg
210 215 220
Leu Arg Gly Gly Phe
225

<210> 81
<211> 345
<212> DNA
<213> Eucalyptus grandis

<400> 81
taataaatga tgaatttatt ataaacgtat ccgtttgaga tttttgtggg tcataggtgt 60
atcaatttga aatctttgat agtaacaaaa ataattttag gtagtttatg tttttcatga 120
tataaacctt gaaagttaat gctactaaat tgttatatat atattaggca aattacaacc 180
ttaatgcaac agttaatgac gtgatactgt tcagattata gatacaatgg ttatccttga 240
atgaataaga agaagtccta agggcaagtg ctatgagctt gcacgactgc ttttgcgcca 300
tttttgttta ccagcccggg ccgtcgacca cgcgtgccct atagt 345

<210> 82
<211> 72
<212> DNA
<213> Eucalyptus grandis

<400> 82
cagtagggga cttgttcccc caagggcacg tgtcgttggt gaagctctgg cggtggatga 60
accgcgtggg cc 72

<210> 83
<211> 544
<212> DNA
<213> Eucalyptus grandis

<400> 83
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taacaaattt atcattcatt aattttcgtt aaattttatt ttcaaattac tgagtcgaat 480
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<210> 84
<211> 515
<212> DNA
<213> Eucalyptus grandis

<400> 84
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ctgaacagta tcacgtcatt aactgttgca ttaag 515

<210> 85
<211> 515

<212> DNA
<213> Eucalyptus grandis

<400> 85
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tatccaggag cgtttgtaca ctgggagtac agagcttctt gcgataccga aactaccctt     180
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cccagagcga agtgtcgatt ttgtccttcc acgaggcttt acctactccc atcgcccgag     300
ccccaagccc aggcccaaat gcctgttcct tgtggccctg ccaacattcc ctttgaaatt     360
aaaaaattaa aaaaaaactc tctgccaggc aaaagtaaag attaacacca ccaaaattta     420
taacaaattt atcattcatt aattttcgtt aaattttatt ttcaaattac tgagtcgaat     480
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<210> 86
<211> 782
<212> DNA
<213> Eucalyptus grandis

<400> 86
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ctcgacggcc tcgacacctc cgccattctc tccgagacca cctccggcgt cgccaagctc     180
atcggcaagc ccgaggccta tgtgatgatt gtgttgaagg ggtcagtccc catggctttt     240
ggtgggactg agcaacctgc tgcctatggc gagttggtgt caatcggcgg tttgaacccc     300
gatgtgaaca agaagctgag tgctgcaatt gcttcaatcc tcgaaaccaa gctgtccatc     360
cccaagtcgc ggttcttcct gaaattttat gataccaagg gttccttctt tggatggaat     420
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taaactctac ttctaggcct gttaaaagtg tcttttttaa ggtaattctg ctattacccc     540
tcttaagtgc atcttatcag taaacatgga atatcctgaa ctttgattat atgccggctc     600
gtggctgtgg aagcacttct ttatgttacc accagcttct caggtgaata taagctttgc     660
ccagtctgtt ctctgggga tttgcttggt gggtagtggc aatcagatgg ttttgtcact     720
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ct                                                                    782
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<210> 87
<211> 115
<212> PRT
<213> Eucalyptus grandis

<400> 87
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Gly Lys Pro Glu Ala Tyr Val Met Ile Val Leu Lys Gly Ser Val Pro
        35                  40                  45
Met Ala Phe Gly Gly Thr Glu Gln Pro Ala Ala Tyr Gly Glu Leu Val
    50                  55                  60
Ser Ile Gly Gly Leu Asn Pro Asp Val Asn Lys Lys Leu Ser Ala Ala
65                  70                  75                  80
Ile Ala Ser Ile Leu Glu Thr Lys Leu Ser Ile Pro Lys Ser Arg Phe
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Ser Thr Phe
        115
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<210> 88
<211> 1521
<212> DNA
<213> Pinus radiata

<400> 88
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tgtcgaatac atcaagcaca tgaagaagca atttgtgcca taggctatct ttagcctcat 180
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<210> 89
<211> 2590
<212> DNA
<213> Eucalyptus grandis

<400> 89
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<210> 90
<211> 1172
<212> DNA
<213> Eucalyptus grandis

<400> 90
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<210> 91
<211> 446

<212> DNA
<213> Eucalyptus grandis

<400> 91
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<210> 92
<211> 2119
<212> DNA
<213> Pinus radiata

<400> 92
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<210> 93

<211> 2571
<212> DNA
<213> Eucalyptus grandis

<400> 93
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<400> 95

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<211> 230
<212> DNA
<213> Pinus radiata

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<212> DNA
<213> Eucalyptus grandis

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<212> DNA
<213> Eucalyptus grandis

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<212> DNA
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<211> 342
<212> DNA
<213> Eucalyptus grandis

<400> 106
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acgagggtcg aatttatagt gggcgaagga tgattaggtg gaatatgaca agaaaatagg 180
tttgaaagag aaataaatat tatgatagtg aagggtcttc acatggttag tttgatctgt 240
ccgagggtgt ccacccttgt ctgatccgca attgctcttg gtcgtgctga attttagagt 300
gtagccaaag taagaatttt cctttcactg tccggacatt tc 342

<210> 107
<211> 948
<212> DNA
<213> Eucalyptus grandis

<400> 107
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aaattcgcac aaccaaagtt gctatgcaga agtcaactca atcctaatca agttgatgag 180
agtgttgggc cctatttttct gcagcaaaca tgaatctcga ttcatctccc tcgcaaaaga 240
taaggaagct gcaaaagctt tcctcctaag tttgttggca agcaaattga ttttgtacca 300
gaaataaata caaagtgaaa cccaagcaat cacgcatggc ctgatttgtg ccatgtccat 360
ttgatctccc tctactattt ttcctgcttt ctcaagcaaa ctagttgctg taacagtgaa 420
tgatccccg gctctctctc tctctctctc tctctctctc catttattcc atccatgttt 480
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ttggaacctc tcacctaatt tcattttctc ccactttgat gagcaccact ctctttccca 600
gatttcaaat aaattgccac tctctccctc ctctttcctc acacaaccaa aagccttctt 660
caagtaccac ttcttcactg tcctctcttc acaatccccc tcttaccaag agcaaagcaa 720
aaaacatgat gaagagactg tcatttctgc tcctactggt cctgctcttc caatgctcta 780
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acatcacgaa ggtcctcaag aaggtgagcc aatttacggt gctgctca 948

<210> 108
<211> 362
<212> DNA
<213> Eucalyptus grandis

<400> 108
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catctctaaa gcagtatgga aaagaaaaca gtccggaagt ctctagctca aaaactgtaa 120

```
ccccgaccta attccggttg tctctgatta catcaattct tatgtcttaa cactccattc      180
gcacctccac aataaataga tcggcccttc atctcccctt accatcgaat ccaatcccaa      240
aaacacttgc tcagacacca tcaaatcctt cgcaaagtct ttttcttaca aaaaacaaac      300
gaaagcaacc atgaagcacc agttcattgt tctggctctc ttattcctca tcaacacagc      360
cc                                                                     362
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<210> 109
<211> 326
<212> DNA
<213> Eucalyptus grandis

<400> 109

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aaaaattaca atcaatggtt atcaatggat gttacaaagg gaggttacat atagaggtta       60
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attttgacgg ttctcttgac tttactatct caacgattac tttatttcat catgttgacg      180
gttgcatcca tgattgttga cttcactttt tgtcgattcc ttcaagctgc tgattcttca      240
agttgccaat aattttattc ataaatgacg aaactctagc ctcatccatt aagtttgtta      300
cttgtccaca ataattaaat tcggta                                           326
```

<210> 110
<211> 296
<212> DNA
<213> Pinus radiata

<400> 110

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ttgttcacac acacgggtct ttatatgacg agtgctgctg cgattataaa tagacggggc      120
aattacaaca aaaactcaca gcatttgaag gaagttggag tggtagagtg agaaatacac      180
agcctaatct gaaggaagtt cgagtaatag agtgagaaat ggatcttctt ctcctcatga      240
tgatgcttgt gatgatgggt gtagcaatgc ctactcattc tcaacaaatc actagt          296
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<210> 111
<211> 723
<212> DNA
<213> Pinus radiata

<400> 111

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tcgatgaagt tcccagagat tgtctcgttc aacgtttcct ctttttttcgg gtcaagtcgg     120
gtacagaaga ccactttctt tacgcggtca agacaccgcc attctcgggt caagtcggga      180
ggtccctcct gctcttcctt tttccaaatc cgtaaaattt acagattttt ttaatgtatg      240
aagcccactt tctttatgcg gttgctccca gtcaagacac cgccattgtt gttcacacgc      300
acgggtcttt atatgacgag tgctgctgcg attataaata gacggggcaa ttacaacaaa      360
aactcacagc atttgaagga agttggagtg gtagagtgag aaatcatttg aagggagttg      420
gagtggtaga gtgagaaatc atttgaaggg agttgagaaa tatattggga atctctcttt      480
tttgcagcaa ttagatcttt cctttaatgc tttgagtggg agaattccga cagagtttgg      540
gaacctctct cttttgcggc aataagttgg agtggtagtt ggagtggtag agtgagaaat      600
acacagccta atctgaagga agttggagtg atagagtgag aaatggatcg tcttcttctc      660
ttcatgttga tgcttgtgat gatgggtgta gcaatgccta ctcattctca acaaatcact      720
agt                                                                    723
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<210> 112
<211> 1301
<212> DNA
<213> Pinus radiata

<400> 112

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ccttccccgt cctaccaaaa cccaaacttc ttgcccgaac tcaccttcta tgtattaatt    180
cttattatta tttaataata ataaatagtt aaacataaat ttataaatta attaattttt    240
atgattttta ttttagttta aaaatgtgac attgttatag attaatgctt atgaacgttt    300
attggccata attaccctaa ttaattataa ttaaaatata tagttataat taaaaaattg    360
tatattttat aaattgaatt aagaatttct gatgatattt catcattcaa ttccatctta    420
tcaaagttag agggaatagt taaccatgta ctagatctat tcatagctaa catttgccaa    480
gttcgtacta ggagacttgg attttttta aaacataatt ttggcagtaa aaagtgaatt    540
ctattgtttt gaaaacaaaa caaaatacag gaagcgtgat tgtggggttg ttgttgaact    600
tgcccgggca aaagaagaat gattagcggt agaggagtta gtagttacgt tcaactaaat    660
gcgtgactaa attatttatc ctccgccatg gaagcaggtg attcacacac aacttgctgc    720
acacattgct ctcaaacctt tcctataaat atccgtagca ggggctgcga tgatacacaa    780
cgcatttaat caaactactt tgattacttt ctgtgggttc tactttcttt gaatagtcag    840
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cgcccaaagt gctgactgcg ccgccaccgc ggagtccctg agcccctgcg cctcggcggt   1020
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cgactgcatc tgcgccctcg tccaatcaac catgcaattg ccttccgaat gcggtcttga   1140
gactcctcag tgcccaagcg actagggtct caagaccgtg actgagtgct ggtttcagag   1200
acagtagaca ttctgcctaa taaatgattg tatgagagct tttatatatg gaattgctca   1260
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<210> 113
<211> 3070
<212> DNA
<213> Eucalyptus grandis

<400> 113

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gataaaaagg tagggagata ggggatctcc ccgtctgatg cctcgggtag gttgaaaata    180
aggcaaaagt tcgccgttga atttgacagc aaaagacacc gtcgttatgc attgcatgat    240
ccattgtacc catgtagggt gaaatcctag agtgaggaga tagtccttta gaaagtccca    300
ttccacccta tcataggctt tctgcatatc cattttaaga acagcccgga attgacgtct    360
acattttctg actttaaatt gatgtagaac ctcttagact attaaaatat tgtcctgaat    420
ttgacgtcca ctgacaaaag cgctttgctc ctggaaaata agtacaggca ggtagggctt    480
aaggcgattg gcaatcacct tagaaatgat cttatatgcg taattacaaa gactgatggg    540
gcggtattgg tctaattgtt caggatgtgg taccttgggt attagggcta tgatggttcg    600
attgagattc ggtggtatga tgccagaatt aaaaaagtgc tgcactgatg agaatagttc    660
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ggccttggta agtcccagtt ggaaagtagc ctctctaact tccttcttgg taacaggagc    780
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caaaatttca ggatcgcgca cccaagcttg attgtcatcc tgcaacatac taatcttgtt    960
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ggcgccaacc cagattgcaa agagcacaaa gagcacgacc caacctttcc ttaacaagat   1260
catcaccaga tcggccagta agggtaatat taatttaaca aatagctctt gtaccgggaa   1320
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cccacaaaag gtcagatgtc atcccacgag agagagagag agagagagag agagagagtt   1440
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tggtgtaggg tccaatattt tgcgggaggg ttggtgaacc gcaaagttcc tatatatcga   1560
acctccacca ccatacctca cttcaatccc caccatttat ccgttttatt tcctctgctt   1620
tcctttgctc gagtctcgcg gaagagagag aagagaggag aggagagaat gggttcgacc   1680
```

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ggctccgaga cccagatgac cccgacccaa gtctcggacg acgaggcgaa cctcttcgcc   1740
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ctcctcgaga tcatggccaa ggacgggccg ggcgcgttcc tctccacggg ggaaatcgcg   1860
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cggctctacg gcttagcgcc ggtgtgcaag ttcttggtca agaacgagga cggggtctcc   2040
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aaagatgcgg tccttgaagg cggaatccca ttcaacaagg cgtacgggat gaccgcgttc   2160
gagtatcatg gcaccgaccc gcgattcaac aagatcttta accggggaat gtctgatcac   2220
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aaaaaaaaaa                                                           3070
```

<210> 114
<211> 1227
<212> DNA
<213> Pinus radiata

<400> 114

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agttattccc ctaatgtgga gacaattgat tagaagttct atgagaaaaa tccaatcatg    180
ttaaagtgac ccctaatgtg aagacaattg attagaaatt ctatgaaaaa aatccaatca    240
tattaaaagt ccaattgatt agcaatttta tgagaaaaat ccaattatgt taaaagtcac    300
tgagtgtggc cgaaattgtg accgaaattg aatgcaataa ccgagggttt ttcaaaccaa    360
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cgataatgga aagcgatatg cagcaaaatc aataaacctg acttcccatg tgagtgatga    540
tttgatcgta caactgatgg tgtgaagtta ctttcagctt caccttcggg cataatcagg    600
gaagtagggc caagtttgct tagtatcact ctaatcccca acaccgtgat tactatcttc    660
atcaacaatg gccaccttcg tcattacttt aactggtggg atacagctac tttacaactg    720
taaatttgtt gaggcagcct atcctcagcc tatacatact aattattgca gctcgattag    780
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atgtggtgaa agtatcgccc atcgtgattc gatggtgtgc agcgagaacg atgatgtatg   1080
taacataaaa tttgaaacag tgcccgacgg actgcctccc caccacgatc gcagtactca   1140
gaatcttgcg gagctcttcc aatccatgga agagaacgct catattcact tccacaagtt   1200
gatggagaag ctccagaatc ttcggga                                        1227
```

<210> 115
<211> 1169
<212> DNA
<213> Eucalyptus grandis

<400> 115
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aaattgagag ggagaatttt agaacaaaat cagatttgga gaatacatgc cattttaggg 180
ggattttggg gatttcgcat atggcgtcgc gtcgtcggcg ccttcttctt tacagattgt 240
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ttcattatat gattattacg tcataatgat cgatttctag aaatttggag acatatgtaa 360
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<210> 116
<211> 947
<212> DNA
<213> Eucalyptus grandis

<400> 116
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acgagggtcg aatttatagt gggcgaagga tgattaggtg gaatatgaca agaaaatagg 180
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cccgtgtcta cccattcttg agaaccttcg agtggaaagc atgaataacc caccttgtac 420
tatataggtt gccgaatatg cctagggcgc gaccatcatt gagacggagt tggggtgctc 480
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agaaggccaa gtccttttct ttatgccgaa gtcctaaagt ttaatatttc aactctacct 840
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<210> 117
<211> 1766
<212> DNA
<213> Eucalyptus grandis

<400> 117
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gtaatatcaa attaaccaga cgataggtgt tgaagattag ctgttgggcc accgtggcga 300

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agttcgtgcg agaaagatca ttttcaatag cataaaagag tctgctttct gctgcaaaca     480
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ctaacaagga ttgatggggg tgaggtgaaa tgtctgtttc ctctttaata tggatccagc     720
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atttctgctc ctactggtcc tgctcttcca atgctctacc accttggctc agcctgcggc    1620
cgccccagct ccgcctgtga tagccccggc tgcacctgct acgcctgcct taggcccggc    1680
tcctcctgtc ttaggcccag ctcctgcagg cccaaccgac atcacgaagg tcctcaagaa    1740
ggtgagccaa tttacggtgc tgctca                                         1766
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<210> 118
<211> 1928
<212> DNA
<213> Eucalyptus grandis

<400> 118

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gttacgttct ccatggaaac tcgaaaggat aaaaattaag aaacggaagc tccatgagaa     180
cgatgggggt cagcatcact cctattgtat tgtgctctca ttatctctgg cctacttgag     240
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<211> 1326
<212> DNA
<213> Pinus radiata

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<212> DNA
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<212> DNA
<213> Pinus radiata

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<212> DNA
<213> Eucalyptus grandis

<400> 123

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<213> Pinus radiata

<400> 124

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<211> 1489
<212> DNA
<213> Eucalyptus grandis

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<211> 1273
<212> DNA
<213> Eucalyptus grandis

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Cys Val Leu Leu Thr Leu Leu Gly Trp Leu Pro Gly Ile Ile Tyr Ala
        35                  40                  45
Val Tyr Ile Leu Thr Lys
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ01/00115

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. 7: C12N 15/11 C12N 15/29 A01H 1/00 A01H 5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
SEE ELECTRONIC DATABASES

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
SEE ELECTRONIC DATABASES

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DGENE, EMBL, GENBANK, SWISS PROTEINS, PIR as per sequence ID Nos specified in inventions 1-5, 12, 20, 23, 30, 33, 36, 37, 39, 45, 46 as stated on extra sheets (1)-(4).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P/X	GenBank Accession No. AR148900. 8 August 2001. See whole document. Also, US6225529 A (PIONEER HI BRED INT) 1 May 2001 See Seq ID 4.	1, 5-17, 23, 24 (Seq ID 59)
P/X	WO 0058474 A (GENESIS RESEARCH AND DEVELOPMENT CORPORATION LIMITED) 5 October 2000. See Table 1 and sequence listing.	1-24 (Seq ID 1-112, 117)
X	GenBank Accession No. AJ012552 (VFA012552). 13 November 1998. See whole document.	2, 3, 5, 9-17 (Seq ID 1)

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2001	19 SEPTEMBER 2001

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GenBank Accession No. L41658 (SCFPOLY). 28 November 1995. See whole document. Also, Albert, H.H. *et al.* 1995. Nucleotide sequence of sugarcane polyubiquitin cDNA. Plant Physiology. 109(1):337-337.	2, 3, 5, 9-17 (Seq ID 34)
X	GenPept Accession No. AAB21993. 7 May 1993. See whole document. Also, Christensen, A.H. *et al.* 1992. Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. Plant Molecular Biology. 18(4):675-689.	4 (Seq ID 67)
X	GenPept Accession No. AAA68878. 23 June 1995. See whole document. Also, Callis, J. *et al.* 1995. Structure and evolution of genes encoding polyubiquitin and ubiquitin-like proteins in *Arabidopsis thaliana* ecotype Columbia. Genetics. 139(2):921-939.	4 (Seq ID 80)
X	EMBL Accession No. D10851 (ATHCDC2BG). 14 April 2000. See whole document. Also, Imajuku, Y. *et al.* 1992. Exon-intron organization of the *Arabidopsis thaliana* protein kinase genes CDC2a and CDC2b. FEBS Letters. 304:73-77.	1, 5-17, 23, 24 (Seq ID 4)
X	EMBL Accession No. U12012 (PTU12012). 23 March 1996. See whole document. Also, Voo, K.S. *et al.* 1995. 4-coumarate:coenzyme a ligase from loblolly pine xylem. Isolation, characterisation, and complementary DNA cloning. Plant Physiology. 108(1):85-97.	1, 5-17, 23, 24 (Seq ID 6)
X	GenBank Accession No. AF139445. 1 June 1999. See whole document.	1, 5-17, 23, 24 (Seq ID 7, 8)
X P/X	Asamizu,E. *et al.* 1998. Structural analysis of *Arabidopsis thaliana* chromosome 5. VIII. Sequence features of the regions of 1,081,958 bp covered by seventeen physically assigned P1 and TAC clones. DNA Research. 5(6):379-391. Also, GenBank Accession No. AB016885. 27 December 2000 See whole document.	1,5-17,23,24 (Seq ID 20)
X	SWISS-PROT Accession No. O24493 (MC1_PINRA). 15 July 1999.	4 (Seq ID 73-75)
X	GenBank Accession No. AF075270. 24 September 1998. See whole document.	1, 5-17, 23, 24 (Seq ID 30)

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GenBank Accession No. X61915 (PTCABP). 23 November 1998. See whole document. Also, Kojima, K. *et al*. 1992. Structure of the pine (*Pinus thunbergii*) chlorophyll a/b-binding protein gene expressed in the absence of light. Plant Molecular Biology. 19(3):405-410.	1, 5-17, 23, 24 (Seq ID 2, 3, 94)
X	GenBank Accession No. U53418 (GMU53418). 28 May 1997. See whole document. Also, Tenhaken, R. *et al*. 1996. Cloning of an enzyme that synthesizes a key nucleotide-sugar precursor of hemicellulose biosynthesis from soybean: UDP-glucose dehydrogenase. Plant Physiology. 112(3):1127-1134.	1, 5-17, 23, 24 (Seq ID 115)
X	GenBank Accession No. Z14990 (ATUBC9). 18 May 1993. See whole document. Also, Girod, P.A. *et al*. 1993. Homologs of the essential ubiquitin conjugating enzymes UBC1, 4, and 5 in yeast are encoded by a multigene family in *Arabidopsis thaliana*. Plant Journal. 3 (4):545-552.	14-17 (Seq ID 50)
X	Walden, A.R. *et al*. 1999. Genes expressed in *Pinus radiata* male cones include homologs to anther-specific and pathogenesis response genes. Plant Physiology. 121(4):1103-1116. Also	1, 3, 5-17, 23, 24 (Seq ID 51, 52, 53, 112)
P/X	GenBank Accession No. U90350 (PRU90350). 17 October 2000. See whole document.	
X	EMBL Accession No. D63396 (NTBY2A, TOBBY2A). 13 February 1999. See whole document. Also, Kumagai F. *et al*. 1995. The involvement of protein synthesis elongation factor 1a in the organization of microtubles in the perinuclear region during the cell cycle transition from M phase to G1 phase in tobacco BY-2 cells. Bot. Acta. 108:467-473.	1, 3, 5-17, 23, 24 (Seq ID 61)
X	GenPept Accession No. AAD56019 (AF181491_1). 22 September 1999. See whole document.	4 (Seq ID 79)
X	GenBank Accession No. X74814 (EGOMTRN). 22 September 1994. See whole document. Also, Poeydomenge,O. *et al*. 1994. A cDNA encoding S-adenosyl-L-methionine:caffeic acid 3-O-methyltransferase from *Eucalyptus*. Plant Physiology. 105(2):749-750.	1, 5-17, 23, 24 (Seq ID 113)
X	GenBank Accession No. X53043 (LEEF1A). 9 May 1995. See whole document. Also, Curie, C. *et al*. 1992. The activation process of *Arabidopsis thaliana* A1 gene encoding the translation elongation factor *EF-1* alpha is conserved among angiosperms. Plant Molecular Biology. 18(6):1083-1089.	1, 5-17, 23, 24 (Seq ID 127)

Box I **Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :

   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos :

   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos :

   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

The international application does not comply with the requirement of unity of invention because it does not relate to one invention only or to a group of inventions so linked as to form a single general inventive concept.
(continued on extra sheet 1)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically:

   Three (3) additional search fees were paid resulting in a total of 15 inventions being searched as follows:
   Inventions: 1-5, 12, 20, 23, 30, 33, 36, 37, 39, 45 and 46 as stated on the extra sheets (1)-(4).

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ01/00115

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: II (extra sheet 1)

The international application has claimed nucleic acid sequences of 46 different regulatory regions, their use in modifying endogenous and/or heterologous gene expression and the phenotypes of plants resulting from this gene expression. Also claimed are coding regions relating to several of the regulatory regions.

The nucleic acid sequences and their putative amino acid sequences have been shown to have a similarity to promoters that are known to be involved in the regulation of transcription and/or expression in plants (p.6 Lines 25-32 and Table 1). Based on this methodology, sequences 1-14, 20 and 22-127 have been assigned with 46 different regulatory activities. However, these regulatory regions and proteins are not unified by a sequence homology or by a common gene upon which they act. Plant promoters generally have been known in the art for some time and indeed many of the promoters referred to in Table 1 have previously been identified and used (refer to citations listed in Box C). Therefore, the use of the nucleotide sequences identified as promoters to modulate transcription in plants does not constitute a special technical feature under Rule 13.2.

The International Searching Authority has found that there are 46 separate inventions, wherein a single promoter or transcription modulator provides the special technical feature; they are listed below:

1. Nucleic and amino acid sequences SEQ ID NOs 1-3, 34, 67, 80 and their at least 40% identical homologues encoding super ubiquitin and regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
2. Nucleic acid sequence SEQ ID NO 4 and its at least 40% identical homologues encoding a cell divisional control regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
3. Nucleic acid sequence SEQ ID NO 5 and its at least 40% identical homologues encoding a xylogenesis specific regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
4. Nucleic acid sequence SEQ ID NO 6 and its at least 40% identical homologues encoding a 4-Coumarate-CoA Ligase regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
5. Nucleic acid sequences SEQ ID NOs 7, 8, 20 and their at least 40% identical homologues encoding cellulose synthase regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
6. Nucleic acid sequences SEQ ID NOs 9-11 and their at least 40% identical homologues encoding leaf specific regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
7. Nucleic and amino acid sequences SEQ ID NOs 12, 60, 78 and their at least 40% identical homologues encoding O-methyl transferase regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
8. Nucleic acid sequences SEQ ID NOs 13, 14, 126 and their at least 40% identical homologues encoding root specific regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
9. Nucleic and amino acid sequences SEQ ID NOs 22, 63 and their at least 40% identical homologues encoding pollen coat protein regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.

(continued on extra sheet 2)

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: II (extra sheet 2)

10. Nucleic and amino acid sequences SEQ ID NOs 23-25, 64 and their at least 40% identical homologues encoding pollen allergen regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
11. Nucleic and amino acid sequences SEQ ID NOs 26-28, 65, 66 and their at least 40% identical homologues encoding auxin induced protein regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
12. Nucleic acid sequences SEQ ID NOs 29-33, 59, 89, 90 and their at least 40% identical homologues encoding flower specific regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
13. Nucleic and amino acid sequences SEQ ID NOs 35, 39, 68, 93 and their at least 40% identical homologues encoding glyceraldehyde-3-phosphate dehydrogenase regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
14. Nucleic and amino acid sequences SEQ ID NOs 36 and 69 and their at least 40% identical homologues encoding carbonic anhydrase regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
15. Nucleic acid sequences SEQ ID NOs 37, 38 and their at least 40% identical homologues encoding isoflavone reductase regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
16. Nucleic and amino acid sequences SEQ ID NOs 40, 70 and their at least 40% identical homologues encoding bud specific regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
17. Nucleic acid sequences SEQ ID NOs 41-44, 92 and their at least 40% identical homologues encoding xylem specific regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
18. Nucleic and amino acid sequences SEQ ID NOs 45, 71 and their at least 40% identical homologues encoding meristem specific regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
19. Nucleic and amino acid sequences SEQ ID NOs 46-48, 72 and their at least 40% identical homologues encoding senescence like protein regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
20. Nucleic and amino acid sequences SEQ ID NOs 49-53, 73-75, 94 and their at least 40% identical homologues encoding pollen specific regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
21. Nucleic acid sequences SEQ ID NOs 54, 55 and their at least 40% identical homologues encoding nodulin homolog pollen specific regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
22. Nucleic and amino acid sequences SEQ ID NOs 56-58, 76, 77, 91 and their at least 40% identical homologues encoding sucrose synthase regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.

(continued on extra sheet 3)

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: II (extra sheet 3)

23. Nucleic and amino acid sequences SEQ ID NOs 61, 62, 79 and their at least 40% identical homologues encoding elongation factor A regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
24. Nucleic and amino acid sequences SEQ ID NOs 81-86, 87 and their at least identical 40% homologues encoding MIF homologue regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
25. Nucleic acid sequence SEQ ID NO 88 and its at least 40% identical homologues encoding a chalcone synthase regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
26. Nucleic acid sequences SEQ ID NOs 95, 96 and their at least 40% identical homologues encoding *Pinus radiata* male specific protein regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
27. Nucleic acid sequences SEQ ID NOs 97, 114 and their at least 40% identical homologues encoding UDP glucose glycosyltransferase regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
28. Nucleic acid sequences SEQ ID NOs 98, 99 and their at least 40% identical homologues encoding elongation factor A1 regulatory and coding regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
29. Nucleic acid sequences SEQ ID NOs 100-102 and their at least 40% identical homologues encoding S-adenosylmethionine synthetase regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
30. Nucleic acid sequences SEQ ID NOs 103, 115 and their at least 40% identical homologues encoding UDP glucose-6-dehydrogenase regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
31. Nucleic acid sequences SEQ ID NO 104 and its at least 40% identical homologues encoding a hypothetical protein regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
32. Nucleic acid sequences SEQ ID NOs 105, 106, 116 and their at least 40% identical homologues encoding laccase 1 regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
33. Nucleic acid sequences SEQ ID NOs 107, 117 and their at least 40% identical homologues encoding arabinogalactan-like 1 regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
34. Nucleic acid sequences SEQ ID NOs 108, 109 and their at least 40% identical homologues encoding arabinogalactan-like 2 regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
35. Nucleic acid sequences SEQ ID NOs 110, 111 and their at least 40% identical homologues encoding root receptor-like kinase regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.

(continued on extra sheet 4)

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: II (extra sheet 4)

36. Nucleic acid sequence SEQ ID NO 112 and its at least 40% identical homologues encoding a *Pinus radiata* lipid transfer protein 2 regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
37. Nucleic acid sequence SEQ ID NO 113 and its at least 40% identical homologues encoding a caffeic acid O-methyltransferase regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
38. Nucleic acid sequence SEQ ID NO 118 and its at least 40% identical homologues encoding a constans regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
39. Nucleic acid sequence SEQ ID NO 119 and its at least 40% identical homologues encoding a flowering promoting factor 1 regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
40. Nucleic acid sequence SEQ ID NO 120 and its at least 40% identical homologues encoding an agamous regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
41. Nucleic acid sequence SEQ ID NO 121 and their at least 40% identical homologues encoding a dreb 1A transcription factor regulatory , DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
42. Nucleic acid sequence SEQ ID NO 122 and their at least 40% identical homologues encoding a drought induced protein 19 regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
43. Nucleic acid sequence SEQ ID NO 123 and its at least 40% identical homologues encoding a salt tolerance protein regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
44. Nucleic and amino acid sequences SEQ ID NOs 124, 130 and their at least 40% identical homologues encoding low temperature induced LTI-16 coding and regulatory regions, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
45. Nucleic acid sequence SEQ ID NO 125 and its at least 40% identical homologues encoding a xylem specific receptor-like kinase regulatory region, DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.
46. Nucleic acid sequence SEQ ID NO 127 and its at least 40% identical homologues encoding an elongation factor 1-alpha regulatory , DNA constructs including same and methods of modulating transcription, expression and phenotype in plants using these sequences.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ01/00115

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
US	6225529	US	6020162
WO	00/58474	AU	00/27024

END OF ANNEX